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MUTATIONS AND REVERSIONS IN REPRODUCTIVITY OF ASPERGILLI WITH NITRITE, COLCHICINE AND d-LYSINE

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Claims for the chemical production of mutants in species of fungi have existed in the literature for many years.[2,5] The major obstacle to their acceptance has been the rarity with which positive results were obtained, hence the failure of subsequent workers in reproducing them. Under these circumstances the mutations described might be considered to have occurred without any specific relation to the experimental conditions set up. The most striking evidence for chemical induction of mutation has been obtained with colchicine in the production of polyploidy in phanerogams,[1] but so far all investigators, including ourselves, have reported negative results with this alkaloid on fungi.

Our first successful use of chemical induction of mutation with fungi was by means of sodium nitrite in acid solution, i.e., nitrous acid. "Injury" mutants in which differentiation, and therefore reproduction, was suppressed in varying degree were readily formed by the action of nitrite. Ease of production and duplication of results with the Aspergilli approached those of a chemical reaction. Positive results were obtained with selected strains of *Aspergillus niger* and *A. Amstelodami*;[4] and were later extended to include *A. variecolor*, *A. fumigatus*, *A. fischeri*, *A. flavus*, *A. alliaceus* and *A. nidulans*.[3] Though the propriety of referring to these artificially produced forms as mutants may be questioned, some evidence was obtained that they persist through the ascosporic stage.

Since the regularity and ease with which mutants were induced by nitrite suggested the smoothness of action of a chemical process, the suggestion was made that the effect of this compound consisted in the destruction of a varying number of free amino groups in proteins included in the hereditary mechanism. Polyploidy, it was considered, was contraindicated by the type of mutants obtained, nor was it believed to explain the uniformity in response to treatment.

We have attempted to submit the hypothesis of amino group destruction to test. Present difficulties prevent cytological studies to ascertain the effect of the nitrite process upon the nuclear mechanism. The fact that these forms are unsuitable for such studies is not the least of these difficulties. Determination of the amino nitrogen content of the fungus proteins might be feasible but also could not be undertaken under present conditions. It seemed probable, however, that if this hypothesis had any basis in fact, the use of other compounds suitable for removal of amino groups should also lead to formation of mutants of the same type.

The compounds selected for trial included ninhydrin, chloramin T., potassium iodide and hexamethylenamine (formin). All are stable in neutral solution but become reactive under the influence of the acidity accompanying growth. The last compound was included because formaldehyde is gradually liberated from it by the steadily increasing acidity in the cultures and would be capable of combining with free amino groups of the fungus proteins. All these compounds gave a sterility type mutant with *A. niger*, the only organism investigated in this respect.

Another test of the hypothesis consisted in an attempt to reverse the results of the process of nitrite treatment. The possibility existed that replacement of amino groups in the proteins of the mutants would, if feasible, lead to reversion of mutants toward the original strains. Two conditions seemed favorable for inducing this reverse mutation. First, the use of high concentrations of d-lysine in the expectation that in some instances the added amino acid would be introduced intact into the modified proteins during their synthesis. This amino acid was chosen because free amino groups in proteins are known to be associated with the presence of d-lysine. Second, the use of solutions of neutral or slightly alkaline reaction containing ammonium and strong reducing agents, on the chance that chemical conditions favorable for the formation of amino acids from corresponding hydroxy or keto acids would promote recovery to the original strain. The organisms tested in this manner included *A. niger* and *A. Amstelodami*. Reversion mutants showing partial to complete recovery in reproductivity were obtained with *A. niger* using d-lysine or sodium thiosulfate; whereas *A. Amstelodami* as yet has given forms showing only recovery of both conidia and ascospores, but of quite different colony appearance. In both instances an excess of calcium carbonate was introduced into the culture medium.

We have at last succeeded in obtaining positive results with fungi through the use of colchicine. The explanation of the many unsuccessful attempts to obtain mutants of fungi with colchicine is believed to have a simple chemical basis. Colchicine is hydrolyzed by acid to form colchicein, which is apparently of little or no effect. The use of acid nutrient solutions, or the free acids formed through metabolism of fungi, would

serve to nullify the presence of this alkaloid. Addition of excess calcium carbonate to the nutrient solution prevents destruction of colchicine apparently, since mutants were readily obtained with *A. fumigatus*, *A. nidulans*, *A. flavus*, *A. fischeri*, *A. variecolor* and *A. alliaceus*. No mutants were obtained with *A. Amstelodami*, or *A. niger*, though indications of a positive action were given by these species also. Concentrations of colchicine greater than one per cent may be found necessary with the latter species, therefore. Whether disturbances in metabolism because of a fixed neutrality are contributory factors remains to be determined.

The types of mutants obtained with colchicine included forms similar to those obtained with nitrite. Intermediate forms were more plentiful, however, and a few forms showing increased fruitfulness were also found. Partial reversion of mutation in *A. Amstelodami* has also been accomplished with colchicine.

The changes in inheritance brought about experimentally in the eight species of Aspergilli with which we have worked are departures from the normal inheritance of these forms that have in some instances proved stable over more than a score of years. These artificially produced strains again could be duplicated in large measure from a collection of forms from natural sources geographically widely distributed and covering a collecting period of more than thirty years. Conidia and ascospores, moreover, if produced at all in these mutants, persisted unchanged from those of the original strains in form and markings. The chemical facts may also be briefly summarized. The action of nitrite results in the formation of mutants in which the ability to differentiate, and therefore to reproduce, are injured in varying degree. Other reagents known to be destructive of the amino group in amino acids and proteins have a similar effect. The presence of high concentrations of d-lysine, the amino acid presumably affected by nitrite, brings about a reversal in mutation. Chemical conditions conducive to re-introduction of amino groups also bring about a reverse mutation.

Nevertheless, the precise nature of the underlying process is by no means certain as yet in the absence of cytological and analytical data. Nor is the formation of similar type mutants by colchicine particularly helpful to the interpretation of amino group destruction. The mode of action of colchicine on fungi itself needs elucidation, since polyploidy need not necessarily or entirely enter into the observed responses with fungi.

It must be considered that nitrite and other destructive agencies acting on amino groups need not necessarily be the only means of bringing about the formation of this type of mutation. Presumably conditions may be brought about under which the metabolic introduction of ammonia to form amino groups is hindered to a greater or less extent.

Specific mention might be made in conclusion of an important implica-

tion that follows from this interpretation of the mechanism of nitrite mutation and of reversion by d-lysine. An important, if not most important rôle is here assigned to d-lysine in the process of differentiation and reproduction, and concomitantly as a possible basis for strain differences in fruitfulness and certain growth characteristics. Progressive increase in sterility in successive transfers of fungi may, in some instances, have its origin in this process, and may be found possible of counteraction in the presence of excess calcium carbonate by means of d-lysine or of ammonium salt and thiosulfate.

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A COMPARISON OF CHROMOSOMAL ABERRATIONS INDUCED BY X-RAY AND ULTRA-VIOLET RADIATION

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The investigations of Stadler and Sprague[1] on maize, following that of earlier workers,[2,3] have confirmed and furthered our knowledge as to the genetical effects of ultra-violet radiation. They showed that like x-rays, ultra-violet radiation could materially increase the mutation rate, but that on the contrary it differed from x-rays in that no increase in the frequency of translocations was found. Besides point mutations affecting seed and seedling characters, numerous entire and fractional endosperm deficiencies were found, being similar in nature, although differing in relative frequency, from those produced by x-rays. Later Singleton,[4] and Singleton and Clark,[5] showed that plants with defective pollen segregations frequently revealed deficiencies that were chromosomal in nature and not simply gene mutations. These deficiencies were cytologically demonstrable at pachytene, and invariably involved the terminal deletion of a chromosome segment. Muller and MacKensie[6] confirmed the results of Stadler and Sprague in so far as translocations were concerned.

The accumulated evidence to date therefore points to a qualitative difference existing between the effects of ultra-violet and x-ray radiation. In all previous ultra-violet experiments, however, examination of individuals for chromosomal changes was made only after many cell generations had intervened since the time of treatment. A direct comparison of the results

of ultra-violet and x-ray radiation, i.e., examination before the intervention of a cell generation between the time of irradiation and that of observations, might serve to settle the question of whether or not a truly qualitative difference does exist between the effects resulting from these two kinds of radiant energy. It is with this purpose in mind that the present study has been undertaken.

A direct x-ray analysis of chromosomal aberrations is a comparatively easy matter because the extreme penetrating power of these rays permits them to reach cells whose division cycle is fairly accurately known and timed,[7] facilitating in this manner the collection of an extensive amount of data before the elimination of non-viable chromosome alterations. A direct analysis of the effect of ultra-violet treatment has not been too successfully carried out to date because of the drastic absorption of the ultra-violet rays by overlying tissues. However, with the development of

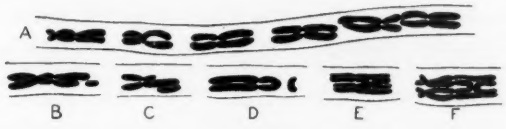

FIGURE 1

Camera lucida drawings of chromosomes in pollen tubes of Tradescantia. Ca. 2000 ×. *A*. Six normal chromosomes in pollen tube. Illustrates manner in which they pass down the tube following acenaphthene treatment. *B* and *C*. Chromatid deletions induced by ultra-violet radiation. *D*. Chromatid dicentric induced by x-rays. *E* and *F*. Chromatid exchanges induced by x-rays.

methods for growing pollen tubes on cultural media,[8] a singularly simple yet effective means is available for carrying out a direct cytological analysis of any chromosomal aberrations or rearrangements induced by ultra-violet radiation (figure 1*A*). In this manner, the generative nucleus can be irradiated in the pollen tube, absorption, therefore, being at a minimum.

The plants used in this study were from a clonal line of a diploid species of Tradescantia. The source of the x-rays was a Coolidge tube equipped with a tungsten target. The source of the ultra-violet radiation was a Hanovia mercury arc lamp operating at 4 amperes and 110 volts d. c. The light was unfiltered, and the treating distance was 10 cm. The heating effect was reduced by the use of an electric fan.

X-Ray Effects on Microspores.—An extensive analysis of x-ray induced chromosomal aberrations in Tradescantia microspores has been carried out by Sax[7] and others. These induced changes fall into two readily distinguishable classes, their nature depending upon the conditions of the

chromosomes at the time of irradiation. Chromosome breaks result from treatment given when the chromosomes are in the form of single threads; chromatid breaks are induced at prophase after the chromosomes have become functionally split into two sister chromatids.

Chromosome breaks are of several kinds, depending upon the number of effectively broken chromosomes concerned. A single break generally involves the deletion of a portion of an arm, the deleted segment lying in the cytoplasm as an acentric fragment. Dicentric chromosomes, ring chromosomes and exchanges between non-homologous chromosomes are also frequently observed.

Chromatid breaks can likewise be classified as to their one-hit or two-hit nature. Single breaks may include one or both of the sister chromatids. If one is broken, this is visible as a shortened chromatid accompanied by a deleted fragment; if both are broken, lateral fusions between the broken ends of sister chromatids give a dicentric chromatid and a U-shaped fragment. Independent hits in two adjacent chromosomes may result in a chromatid exchange; if in the same chromosome, a ring will be produced.

X-Ray Treatment of Dry Pollen Grains.—Open flowers were given doses of 200 r, and the pollen grains germinated on a cultural medium. The data are given in table 1.

TABLE 1

X-RAY DATA ON POLLEN GRAINS IRRADIATED DRY. 200 R

Figures represent number of chromosomes examined

NORMAL	CHROMATID DELETIONS	CHROMATID DICENTRICS	CHROMATID EXCHANGES	DICENTRIC CHROMOSOMES	TOTAL	% BREAKS
979	4	15	2	2	1002	2.29

Chromosome changes induced at this stage of pollen maturity are very similar in nature to those resulting from irradiation of early prophase nuclei in the microspores. The presence of two dicentric chromosomes indicates that the chromosomes of the generative nucleus at the time of pollen maturity are not all effectively split into sister chromatids. Dicentric chromosomes are never found when microspores are irradiated in prophase, so that their presence in the pollen tube is a valid criterion of the singleness of some of the chromosomes.

X-Ray Treatment of Pollen Tubes.—Pollen tubes were subjected to x-rays at approximately two hours after germination. As will be seen later, this particular time for irradiation was chosen so that a strictly comparable set of data might be obtained to test for the presence or absence of a qualitative difference between the effects of x-ray and ultra-violet radiation on chromosome breakage. The dosage was 240 r. The pollen tubes, however, were irradiated while still in the glass moist chamber in which

they were growing so that the actual dosage reaching the nuceli was somewhat lower due to partial absorption of the rays by the glass. The data are given in table 2.

TABLE 2

X-RAY DATA ON POLLEN TUBES. 240 R

NORMAL	CHROMATID DELETIONS	CHROMATID DICENTRICS	CHROMATID EXCHANGES	TOTAL	% BREAKS
946	29	22	5	1002	5.58

Under this treatment, chromatid deletions are more numerous than chromatid breaks (figure 1*D*), although both are undoubtedly the result of single quantum "hits." The high percentage of deletions is considerably above that found in x-rayed microspores whether rayed in prophase or resting stage, Sax[7] reporting that only five per cent of the aberrations induced at resting stage are of this type as opposed to over fifty percentage here. It is quite probable that an explanation may be sought in the spatial relationships of the sister chromatids at this time. The threads are without doubt farther apart when the generative nucleus has passed out into the tube than when it lies passively in the ungerminated pollen grain. Also a considerable tension is exerted on the chromosomes as they pass down the tube. This may be deduced from the fact that frequently the nucleus becomes separated into two or more independent groups of chromosomes, and also from the fact that occasional chromosomes are stretched almost to a breaking point as they move downward. This tension is probably set up by rapid protoplasmic streaming. Internal movements in the individual chromosomes as the result of prophase contraction may also be another factor in keeping broken ends from refusing into their original positions once they have become separated from each other. If this be the case, we might expect that the number of deletions found in irradiated pollen tubes reflects more nearly the actual percentage of breaks induced by this treatment than does the observed percentage of breaks in the microspores, where, according to Sax,[7] the frequency of actual breaks undoubtedly is much greater than the observed frequency, the majority of them reuniting back into their original positions.

In addition, chromatid exchanges (figure 1*E*, *F*) between non-homologous chromosomes are also present. No dicentric chromosomes were found. Evidently completion of the effective splitting had taken place by this time, and all aberrations could be classified as chromatid breaks.

Ultra-Violet Treatment of Pollen Tubes.—Pollen grains were germinated on slides and the generative nucleus irradiated two hours after sowing. Exposure was for 30 seconds. Longer exposures were found to be inadvisable because the film of medium soon becomes desiccated, inhibiting

the growth of the pollen tubes and finally causing death. Three different groups of slides were analyzed (table 3).

TABLE 4

ULTRA-VIOLET DATA ON POLLEN TUBES

30-second exposures at distance of 10 cm.

NORMAL	CHROMATID DELETIONS	CHROMATID DICENTRICS	TOTAL	% BREAKS
(*a*) 2155	23	0	2178	1.05
(*b*) 1273	11	0	1284	0.86
(*c*) 1565	11	2	1578	0.82
Con. 1587	2	1	1590	0.18

Experimental evidence reveals that the chromosomal aberrations induced by ultra-violet radiation are almost exclusively of the chromatid deletion type (figure 1*B*, *C*). So far as could be determined cytologically all deletions were terminal in nature, and of varying length, the breaks not being localized in any particular regions. All gradations from free fragments to achromatic lesions were found. However, only those breaks which unquestionably showed broken ends were considered in this study, although some of the achromatic lesions were undoubtedly true deletions which had not as yet become separated from the remainder of the chromosome. No lesions were found that extended across both chromatids at the same locus.

Only two chromatid dicentrics were observed in the ultra-violet treated material (figure 1*D*). Unlike the deletions, these aberrations involve breakage of both chromatids at the same locus, followed by lateral fusion to give the dicentric chromatid plus the U-shaped fragment. One of the two breaks lacked the usual fragment, and an examination of the entire length of the pollen tube and grain failed to reveal it lying in the cytoplasm. Evidently the above dicentric chromatid was the result of some previous microspore aberration which had been carried over into the generative nucleus. The only other chromatid break was similar to that found in the control, and probably was of spontaneous origin.

Discussion.—It has thus been possible to obtain a direct analysis of ultra-violet induced chromosomal aberrations, confirming in this manner the genetical results of Stadler and Sprague[1] and of Muller and MacKensie.[6] Cytologically observable deficiencies occur, but no increase in the percentage of translocations was found. The fact that a single effective ultra-violet "hit" can only break a single thread at any one locus while a single x-ray "hit" can break both threads seems best explained by assuming that the sphere of influence of an absorbed ultra-violet quantum is an area no greater in diameter than the width of a single chromatid, while that of an x-ray quantum is sufficiently wide in extent to include both. On the basis of energy values this becomes explicable, for as Good-

speed and Uber[9] point out, the energy of an ultra-violet quantum is from 4 to 6 electron-volts, whereas that of an x-ray quantum may range from 10,000 to 200,000 or more, the value increasing inversely as the wave-length.

The difference in the width of this so-called "sphere of influence" may perhaps be thought of as an expression of the difference in the physical behavior attending the absorption of these quanta. The curves obtained when breaks[7] or survival ratios[10, 11] are plotted against dosage imply the effectiveness of single quantum absorptions. It is now well established that the ionization created by x-rays is responsible for the chromosome breakage. Ultra-violet, on the other hand, because of the low energy values of a single quantum, cannot create a path of intense ionization. Instead, it derives its effectiveness from the fact that it can excite the absorbing molecules to higher energy states by affecting the electrons in only the outer orbits. Ultra-violet has, on this account, been frequently spoken of as "chemical rays." This ionization is therefore highly localized, and does not extend across the distance between the sister chromatids to cause a similar reaction at the same locus in the other thread.

This, however, does not explain the absence of translocations under ultra-violet treatment. The question arises as to whether or not chromosome breaks induced by ultra-violet radiation are capable of reuniting back into their original positions or into new associations. Sax[12] has shown that x-ray induced breaks can remain in an unstable condition and capable of re-fusion for as long as an hour after the time of irradiation. Does the absorption of an ultra-violet quantum leave a broken end in such a labile condition, or does the chemical action of these rays leave a satisfied bond at the broken end such that re-fusion is impossible? For the present this question must remain unanswered, but its solution might serve to explain the absence of gross chromosomal rearrangements with ultra-violet treatment under circumstances where comparable doses of x-ray, as judged from the frequency of endosperm deficiencies[1] and lethals,[5] produce an abundance of these types of aberrations.

Ultra-violet radiation of maize pollen[1] yields two kinds of endosperm deficiencies, entire and fractional, as judged by the absence of dominant endosperm characters. The fractional deficiencies generally consist of kernels showing 1/2 of the tissue recessive and the other 1/2 dominant, with about equal numbers of larger and smaller proportions. If we assume that the aberration induced by the treatment consists of the deletion of a segment of the chromosome arm when the chromosome is in the two-thread state, the production of fractionals becomes readily understandable. This is indicated by Stadler and Sprague's data which show that losses of *C* and *Wx* are usually associated, eliminating the possibility that these are simple point mutations. The first division of the endosperm-

fusion-nucleus, resulting from fertilization by such an altered sperm cell, would therefore give one cell with a normal, and one with a deleted, chromosome. The amount of endosperm derived from each of these cells would be approximately equal, giving kernels chiefly of the "1/2" class, but if the products of the two cells were unequal, as well might be the case since division in the endosperm is an irregular process, then equal numbers of larger and smaller proportions would be expected.

The production of entire endosperm deficiencies by ultra-violet treatment is not so easily explained. There are four possible ways by which this might be brought about: (1) ultra-violet induces breaks in both chromatids of a single chromosome arm by two independent absorptions, although on a random basis this seems very unlikely if all breaks are simple deletions; (2) a single "hit" can induce a chromatid break involving both chromatids at the same locus, a situation not yet demonstrated cytologically; (3) a single quantum might be absorbed in the region of the centromere where the thread is effectively single although the chromatids are split, giving a deletion for both of the chromatids of an arm; (4) if all of the chromosomes in the sperm nuclei or generative nucleus are not effectively split at the time of irradiation an opportunity is provided for a single "hit" to delete the entire end of a chromosome arm, thus giving an endosperm-fusion-nucleus deficient for that particular segment. At present this explanation seems to be the most satisfactory, and is not without some experimental evidence, for, as has been pointed out above, the appearance of dicentric chromosomes in pollen grains irradiated dry is good factual evidence for the presence of single threads at the time of irradiation. Furthermore, in maize, where division of the generative nucleus to give two sperm cells takes place in the grain instead of in the pollen tube as in Tradescantia, the possibility of having single threads in the sperm nuclei is obviously greater than in the generative nucleus of Tradescantia since no further division of the sperm nuclei takes place before fertilization.

X-ray induced endosperm deficiencies[1] are generally of the "entire" kind, while those induced by ultra-violet are largely fractionals. The experimental data obtained in this study are in good agreement with these conclusions since the chromatid deletions resulting from ultra-violet radiation would give genetically observable fractional deficiencies while the greater proportion of chromatid dicentrics induced by x-rays would result in entire endosperm deficiencies.

Summary.—X-ray radiation of the generative nucleus in Tradescantia pollen grains reveals that most of the chromosomes are effectively split into sister chromatids. A great proportion of the chromatid aberrations involve deletions of both chromatids of a chromosome at identical loci, thus confirming the genetic data in maize in respect to endosperm deficiencies. Irradiation of the generative nucleus in the pollen tube results

in only chromatid aberrations including a considerable number of simple chromatid deletions and occasional chromatid exchanges.

Ultra-violet radiation of the generative nucleus in the pollen tube induces only simple chromatid deletions. The loss of only one of the two chromatids is in accord with the genetic observations that ultra-violet radiation produces primarily fractional endosperm deficiencies in maize. No configurations representing an interchange of chromatin between non-homologous chromosomes were found.

The qualitative difference between the types of breaks induced by x-ray and ultra-violet radiation is tentatively explained by assuming that the sphere of influence of a single x-ray quantum is much greater in area than that of a single ultra-violet quantum. The vast difference in energy values, and the difference in the physical behavior attendant to absorption of the respective quanta supply a possible physical and chemical basis for this variation in degree of effectiveness.

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CYTOLOGY AND DEVELOPMENT OF THE EMBRYOS OF X-RAYED ADULT DROSOPHILA MELANOGASTER

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In 1927 Muller[1] conclusively demonstrated that mutations could be induced in animals by means of x-rays. Exposure of the germ cells of *Drosophila melanogaster* to relatively heavy doses of x-rays resulted in the production of large numbers of mutations of different types, including recessive lethals, semi-lethals and visibles of various kinds. "In addition, it was also possible to obtain evidence in these experiments for the first time, of the occurrence of dominant lethal genetic changes, both in the X

and in the other chromosomes. Since the zygotes receiving these never developed to maturity, such lethals could not be detected individually, but their number was so great that through egg counts and effects on the sex ratio evidence could be obtained of them en masse." Dominant lethals are, thus, a class of lethals which can effect the death of zygotes even though present in single dose, i.e., introduced by but one of the gametes.

Hanson,[2] shortly thereafter, subjected Drosophila males to x-rays and made counts to determine the extent of the effects of the radiations. Mortality of the F_1 eggs, larvae and pupae when compared to the death rate among the controls was found to be significantly increased. He inferred that dominant lethal mutations had been induced in the treated sperm, resulting in death of the zygotes at various stages in their ontogeny. Timofeef-Ressovsky,[3] Gowen and Gay,[4] Sonnenblick,[5] Demerec, Kaufmann and Hoover,[6] Kaufmann[7] and others have also noted the marked increase in mortality among the progeny of irradiated adult Drosophila. Furthermore, the induction of non-transmissible dominant lethals in Habrobracon has been reported.[8, 9]

Although the progeny of treated Drosophila die at various levels of development, the high death rate among the embryos is especially striking. In those zygotes which initially commenced development with one haploid complement of untreated chromosomes and with one irradiated chromosomal complement, the majority of the inviable alterations and rearrangements introduced by the treated gametes are thus eliminated prior to hatching of the young larvae. This report, based on egg counts and the study of preparations of approximately one thousand eggs and embryos, will indicate the nature of the inviable chromosomal alterations and their effects on embryonic development.

A wild type Oregon-R strain of *Drosophila melanogaster* was used in the experiments. Tests with this vigorous stock indicate that more than 96 per cent of the deposited normal eggs hatch as larvae. But 55 of 1420 zygotes deposited by untreated parents failed to emerge from the embryonic envelopes. Five-day-old virgins were employed throughout all of the work. The flies were put into small gelatin capsules which were placed on a sheet of cardboard at a distance of 34 cm. from the tungsten target. The tube was operated at 200 kv. and at 30 ma. current. Under these conditions, the output of the machine, as measured in air, was 390 r per minute. Dosages ranging from 195 r to 4680 r were used. The various exposures were given by stopping the machine at measured intervals and removing the samples in sequence. Males and females, in separate capsules, were simultaneously subjected to the radiations and, following the exposure, mated to untreated virgins. Eggs were collected on the moist inside surface of ripe banana skins and at timed periods of development

were fixed and sectioned, securing organisms which were representative of all levels of embryonic development for comparison with controls.

I am greatly indebted to Dr. Paul S. Henshaw, formerly of the Memorial Hospital, New York City, and now at the National Cancer Institute, Washington, D. C., for the x-ray treatments.

Results: (*a*) *Egg Mortality Counts.*—The testes of adult males carry an abundant supply of sperm[10] which are passed to the mates during copulation. Thus, radiation of the males involves exposure of gametes which had already undergone maturation. In the females, however, the situation is different. The physiological condition of the chromosomes at the time of exposure is not like that obtaining in the males. With the ex-

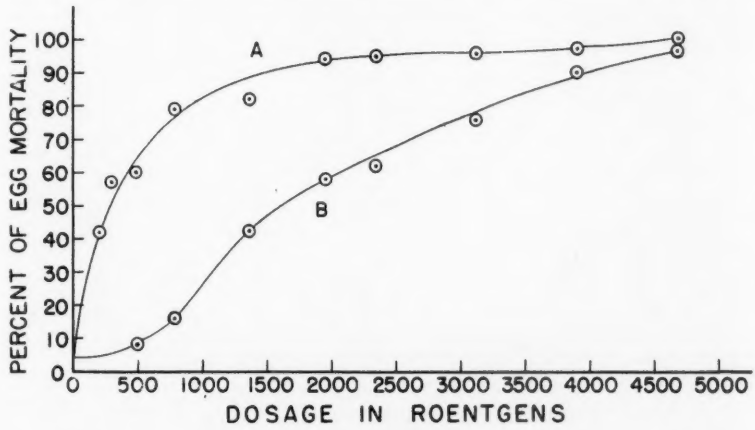
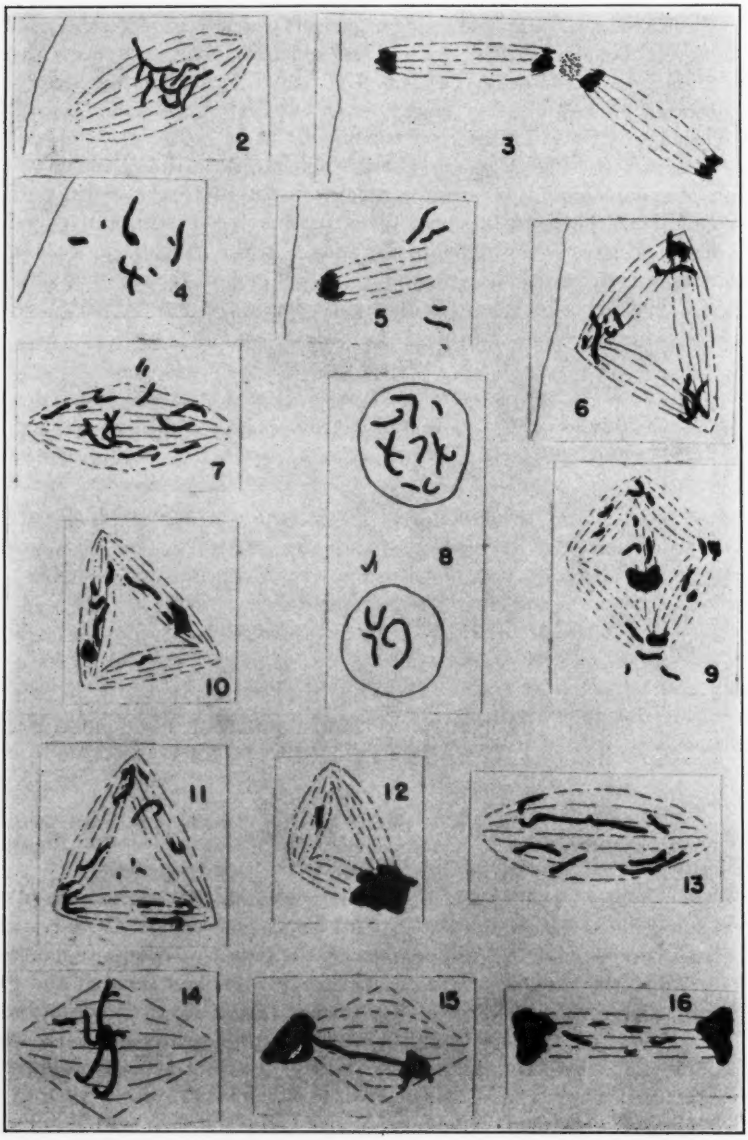


FIGURE 1

Relation between dosage and the percentage of egg mortality. (*A*) Adult females irradiated. (*B*) Adult males irradiated.

ception of the one egg which may be present in the uterus, the other components of the ovarian strings are in a stage not further advanced than metaphase of the initial meiotic division.[11] Following exposure, therefore, the oöcytes and oögonia have yet to pass through the two maturation divisions. Since almost all of the eggs used in this study were collected during the twenty-four-hour period after treatment (a few were collected on the second day), such eggs were the most mature, i.e., closest to first meiotic metaphase, when exposed to the radiations.

Eggs were collected as described above and counts were made in order to determine the number which eventually hatched as larvae. The data are given in table 1, while in figure 1 the percentage of egg mortality is shown plotted against dosage in roentgens. The curves suggested by the



FIGURES 2–16

2, 3. Normal metaphase and telophase of first and second meiotic divisions.
4, 5, 6. Aberrant meiotic figures in eggs deposited by treated females.

points are non-linear and indicate the increase in egg mortality with increasing dosage. The *Y* intercept is at 3.9, representing the average egg mortality in the controls.

There is a decided difference in the sensitivity of the sperm and mature oöcytes to the radiations. The effects of small dosages on the female gametes are very striking. This is evident in the graph, for with dosages up to 780 r the curve *A* rises steeply; from this point the slope diminishes and the curve gradually approaches the maximum. These data follow closely those of A. R. Whiting[12] who irradiated Habrobracon females and determined from daily egg counts that mortality was highest for eggs laid on the first day following the treatment. Curve *B*, representing the variations in egg mortality following exposure of the sperm, has a gradual slope and only at the highest dosage tends to approximate the other curve. The number of affected male gametes, while not directly proportional to dosage, increases gradually.

TABLE 1

	ADULT FEMALES IRRADIATED (X UNTREATED MALES)			ADULT MALES IRRADIATED (X UNTREATED FEMALES)		
DOSAGE IN ROENTGENS	EGGS DEPOSITED	EGGS NOT HATCHING	% EGGS NOT HATCHING	EGGS DEPOSITED	EGGS NOT HATCHING	% EGGS NOT HATCHING
195	458	192	42.1	....	...	....
293	752	432	57.4	....	...	....
488	1080	656	60.7	444	36	8.1
780	564	448	79.4	296	48	16.2
1365	538	454	82.2	498	212	42.5
1950	568	536	94.6	620	360	58.1
2340	772	734	95.1	740	460	62.1
3120	552	528	95.7	1224	928	75.8
3900	530	516	97.4	594	528	88.9
4680	144	144	100.0	251	242	96.5

(*b*) *The Maturation Divisions.*—Entrance of the sperm normally induces the completion of the first meiotic spindle. The chromosomes on the first polar spindle advance as far as metaphase and then pause until the sperm enter the egg, whereupon the divisions proceed. The anastral maturation divisions occur close to the dorsal surface of the egg in an island of protoplasm situated approximately one-fourth of the distance back from the anterior end. The chromatic complexes which separate during the first meiotic division form no vesicular nuclei, but, after a few minutes pass directly upon the second polar spindles (Figs. 2 and 3). The second division proceeds, resulting in four haploid nuclei of which the innermost becomes the female pronucleus. The other three are the polar body

FIGURES 2-16 (*Continued*)

7–16. Types of cleavage figures observed in eggs with treated chromosomes. Fragmentation, chromosome clumping, multipolar configurations are commonly seen. Similar aberrations are noted whether sperm or egg contributes the irradiated chromosomes.

nuclei; these are not extruded from the egg, but remain in the antero-dorsal island and there gradually disintegrate.

In the sections of eggs laid by treated females and fixed between five and sixteen minutes after deposition, various distortions of the maturation figures can be observed. No achromatic figure (Fig. 4) may be present, while the fractured chromosomes are scattered about in the clear protoplasmic island. That this egg was in the stage of polar body formation is indicated by the presence of an unresolved sperm head with its centriole and delicate sperm tail in another section. Occasionally a part of the spindle is missing. The remainder may not have formed at all or, having formed, disintegrated earlier. For example, in figure 5 only a half-spindle with a chromosomal mass at the remaining pole is present; several chromatic elements are lying free in the cytoplasm. In the treated material multipolar spindle configurations rather than the normal bipolar arrangements are sometimes present (Fig. 6). No sperm tails were seen in the island in which the division represented by figure 6 was occurring, thus precluding the possibility that a supernumerary spermatozoon may have established an independent spindle which combined with the first polar body spindle to upset the process of meiosis. The chromosomes and chromosome fragments are distributed over all three poles and it is difficult to establish whether the complex at any one pole is a balanced one. While the maturation divisions are occurring, one of the sperm heads normally becomes large and vesicular and eventually fuses with the resulting female pronucleus. However, if as a result of aberrant divisions no female pronucleus is formed, the sperm head may resolve itself into its haploid complement of chromosomes, but no instance was ever observed in which a haploid sperm complement formed a mitotic figure and began to cleave. In one hundred preparations of early normal eggs, selected at random, only one aberrant meiotic figure was noted. Within the sections of sixty-nine organisms whose mothers had been treated with 2340 r, there were, on the other hand, sixteen with various meiotic abnormalities.

(*c*) *The Early Cleavages.*—During the early cleavages, the egg is a syncytium with the nuclei uniformly scattered throughout the cytoplasm. The nuclei divide synchronously and, at the eighth cleavage, begin their migration to the periphery, arriving in the posterior polar plasm slightly prior to their appearance in the other portions of the egg. Globules of posterior polar plasm, each containing a nucleus and a number of the polar granules, are pinched off; these are the primordial germ cells.

In these normal eggs the mitotic figures are bipolar and equivalent chromosomal groups separate at anaphase. This orderly mode of mitosis is frequently found upset, however, in zygotes developing with treated chromosomes.[13] In such zygotes, chromosome fragmentation, chromatin bridges, asymmetrical divisions, clumping of the chromosomes, multipolar

figures of the tripolar and quadripolar types and cytoplasmic vacuolization have been observed. Within the sections of a single egg, there may be found chromatic and achromatic aberrations of various kinds, as well as apparently normal figures. Furthermore, similar aberrations appear in the eggs, regardless of whether sperm or ovum contributed the irradiated chromosomes. It would be impossible to indicate all of the abnormalities noted in the zygotes and the varied modes of interference with the progress of normal development. Therefore, a representative group has been selected for discussion.

A first cleavage figure, with no evidence of gonomery, is indicated in figure 7. Fracture of one or more chromosomes had occurred, and elimination of some fragments appears to be taking place, since some are off the spindle and lying free in the cytoplasm. Other fragments are passing to the poles and may be incorporated in a daughter nucleus. In a slightly later developmental stage, late prophase of the second division, the nuclear membranes are beginning to disappear but the chromosomes are distinct (Fig. 8). An asymmetrical distribution had evidently occurred during the progress of the first division, for the volume of chromatin present in one of the nuclei is definitely greater than in the other nucleus. Lying close to the lower figure are some fragments which stained lightly and which had not been included in the reconstruction nucleus.

Irregularity in the mechanics of mitosis is well manifested through the frequently observed multipolar figures which are found in the sections of eggs of treated parents. Several of these atypical spindle arrangements may be observed within one egg. The chromosomes and fragments are usually found distributed over the various spindles (Figs. 9, 10 and 11), but are also observed clumped at one of the poles (Fig. 12). With the exception of a rod-like fragment, all of the chromosomes in the latter figure are massed at one pole, forming an irregularly shaped clump which stained intensely. Clumping of the chromosomes is often noticed in the experimental material. The radiations alter the consistency of the chromosomes and the change is usually manifested at anaphase when the viscid strands are about to separate. The pycnotic masses take various shapes (Figs. 12, 13, 14, 15 and 16). At times the clumped masses may be connected by chromosomal strands, but often they appear as more or less solid complexes in which the identity of the individual components is lost. It has not been possible to determine whether the complexes at the poles in figures such as have just been referred to can continue to divide. If restitution nuclei do form and cleavage does continue, nuclei with varying chromosome combinations would result.

Indication has already been given above that irregularities in the mitotic mechanism are by no means restricted to one particular type in any one organism. A tabulated presentation of the occurrence of such

abnormalities in five organisms which were fixed during the early cleavage divisions is given in table 2. It is interesting to note that among the degenerate aberrant figures lie some which are apparently normal and appear to be capable of continuing to divide.

TABLE 2

SEX OF TREATED PARENT	DOSAGE	NUMBER AND TYPE OF MITOTIC ABNORMALITIES
Male	3900 r	11 figures containing clumped chromatic masses; 1 quadripolar figure
Male	3900 r	3 figures with fractured chromosomes; 1 normal figure
Male	3120 r	1 tripolar figure; 3 figures with clumped masses; 1 normal figure
Female	2340 r	3 figures with chromosomes and fragments
Female	2340 r	2 figures with chromosomes and fragments; 1 normal figure

(*d*) *Later Embryonic Stages.*—Development and morphogenesis proceed at such a rate in Drosophila that after twelve hours of incubation at 25°C. the egg has passed from first cleavage to a relatively highly complex, differentiated organism. During this period, the syncytial cleavages occur, the primordial germ cells are segregated, the malpighian tubules and nervous tissue have made their appearance, metamerism has occurred, the definitive number of salivary gland cells is present, the major portion of the yolk has been enclosed in the mid-gut and a pair of gonads are in their permanent position in the postero-dorsal region of the embryo. Later, other structures appear and during the twentieth and twenty-first hour of development a majority of the young larvae emerge from the embryonic cases. Among the offspring of treated adults are found organisms which apparently differ in no morphological aspect from controls of similar age, together with embryos in which extensive cellular proliferation but no differentiation has occurred. Although the latter are from twelve to sixteen hours of age, no sign of structural organization is noticed. Apart from the yolk inclusions which are concentrated in one or another region of the organisms, these embryos consist of massed aggregates of cells. The cells are of various sizes, often vacuolated and their membranes are frequently not intact, resulting in syncytial-like patches. The cells have no orderly arrangement, but are haphazardly dispersed throughout the cytoplasm. No embryo comparable to these was observed in the controls.

Eggs which had not hatched by the third day following deposition, i.e., some forty-eight hours past the normal modal hatching time, were selected at random and dechorionated. In some of the dechorionated eggs no evidence of development could be seen, but in others embryonic differentiations were visible. These embryos were segmented and larval jaws and tracheae were noted. When removed from the membranes, such embryos would sometimes move their jaws and muscles feebly; others remained in the position in which they were placed and showed no activity at all. In

several instances, the larvae had difficulty in emerging wholly from the egg cases, for the envelopes still enclosed the posterior portion of these organisms. Careful removal of the membranes did not induce such individuals to move or show any other activity.

Summary.—When Drosophila males and females are exposed to x-rays and then mated with untreated flies, a high percentage of the embryos do not hatch as larvae. Especially susceptible to the radiations are the most mature oöcytes. The treatments have affected the gametes to an extent that, in many cases, orderly development becomes impossible. Varied distortions of the meiotic and mitotic divisions have been observed. Within the sections of single eggs, distortions of various types as well as apparently normal figures have been found. At an age when normal embryos are highly differentiated, some of the embryonic offspring of irradiated parents consist of structureless masses of cells. It is likely that there is a direct relationship between the observed cytologic and developmental abnormalities and the greatly increased percentage of mortality among the progeny of treated adult Drosophila.

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ON THE LAW FOR MINIMAL DISCRIMINATION OF INTENSITIES. IV. ΔI AS A FUNCTION OF INTENSITY

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Communicated May 7, 1940

The nature of the functional dependence of the sensorially least discriminable increment of intensity (ΔI) upon the magnitude of the prevailing intensity level (I_1) has long been recognized as presenting a problem of fundamental interest. The literature concerning it, experimental and theoretical, is full of tantalizing curiosities, and is voluminous to the point of generating stupefaction. Although enormous labor, in the aggregate, has been directed toward establishing a rational basis for the data of differential sensitivity, no even approximately satisfactory solution has been proposed thus far. The chief reason for this is to be found, I suspect, in the curious fascination of the "Weber fraction" $\Delta I/I$. The intensity of Fechner's realization of the psychophysical significance of the properties of ΔI largely succeeded in fastening attention on the seductive possibilities supposed to follow upon the fact that the "Weber fraction" might be (or "ought" to be) a constant. The chief consequence of this was that the academic and essentially artificial problem of the constancy or inconstancy of the ratio $\Delta I/I$ came to dominate a very considerable area of psychophysics.

A certain degree of sanity was introduced into this matter by Hecht's insistence[1] that the more realistic problem, and the theoretical opportunity, is found not in the "constancy" of $\Delta I/I$ but in the reasons for its systematic behavior as a function of intensity. The rational theory of intensive discrimination has continued to be very largely occupied with the consideration of the properties of $\Delta I/I$[2,3] or sometimes of $I/\Delta I$.

The reasons leading to a rejection of these considerations are of two general sorts. I am referring to the rejection of what I have just termed the current rational theory of intensive discrimination; I am not discussing those attempted formulations of the data which depend only on the use of more or less convenient but not experimentally supported equations, because any number of such formulations can be found and consequently no one of them has of itself any real analytical significance; moreover, none of them thus far detected actually describes the data or takes adequate account of their known properties. The current rational theory of intensive discrimination has had a considerable (although not a completely inclusive) success in giving descriptive formulae for the dependence of visual $\Delta I/I$ upon I. The data of visual intensive discrimination cover a wider range of intensities, in more different kinds of organisms, than are

available with other types of sensory excitation; and they are in some important respects less complicated by the mechanical conditions of sensory reception. For reasons which Hecht has discussed in detail[4] it has been attractive to suppose[3] that the quantity ΔI is (visually) one which produces a certain amount or rate of decomposition of photosensory receptor substance in a system adapted to I_1. The main reasons for rejecting this approach are (1) that there is no necessity for assuming that the magnitude or the properties of ΔI are determined peripherally in the receptor,[5] and

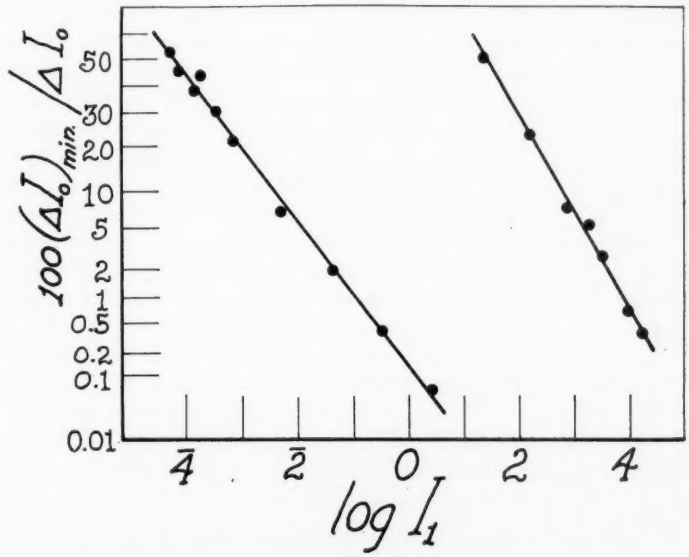

FIGURE 1

"Instantaneous" thresholds (ΔI_0) under conditions of adaptation to intensities I_1: "white" light data from Blanchard;[12] 5° field. At the left, "rod" curve, asymptotic minimum $\Delta I_0 = 0.00589$; at the right, "cone" curve, minimum $\Delta I_0 = 0.100$. The ordinates give $1/\Delta I_0$ as percentage of the maximum value of this fraction, on a probability grid.

(2) there is no reason whatever to accept the assumption that the threshold effect is a physically constant effect.[6] While the resulting equations provide a certain (but incomplete) description of the data, this is surely no argument for their uniqueness and does not by itself validate the assumptions from which they are derived.[7]

The matter may be approached in a quite different way, with the help of simple assumptions for which there is now considerable direct support of a totally different sort. These assumptions avoid most, if not indeed

all, of the general difficulties already referred to. The chief assumptions are: (1) the relation of sensory effect E to $log\ I$ is given by a normal probability integral; the various aspects of "effect produced" as a function of intensity, when objectively measurable, do adhere to this formulation,[7,6] and the reason for it is deducible in an elementary way;[6] (2) the reciprocal of the exciting intensity measures the capacity to be excited under the

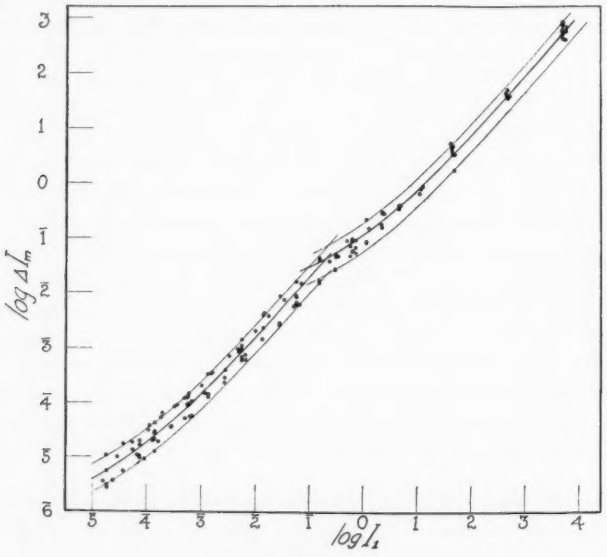

FIGURE 2

Data (cf. [18]) from experiments (Crozier and Holway) by the method of increasing I_1 to I_2, where $I_2 - I_1 = \Delta I$. Each point is the mean of five measurements (left eye, W. J. C., white light, field 30° square centrally fixated; for observational conditions cf.[5]). The central lines are probability integrals for $1/\Delta I_m$ as a function of $log\ I_1$. The statistically constant vertical width of the band corresponds to the fact that $\sigma_{1\Delta I}$ and ΔI_m are in direct proportion.[10] For the lower ("rod") segment, asymptotic minimum $log\ \Delta I_m = \bar{6}.53$; for the upper segment, minimum $log\ \Delta I_m = \bar{3}.80$.

conditions and with reference to the end-point of effect employed;[6,8] the types of justification for this assumption are numerous, and include especially the rational behavior of the three parameters of the probability summation involving $1/I$, under various experimental treatments.[9] Viewed in this way the derivative curve of $dE/d\ log\ I$ as a function of $log\ I$ is of course a frequency distribution of elements of neural effects (and not of the intensity thresholds of excitable neural units).

If an assemblage of neural units is adapted to an intensity I_1 the corresponding mean total sensory effect is E_1. Excitability with respect to the production of any further sensory effect by increasing I must clearly depend on the range of the distribution of $d\ log\ I$ still open to activation, and the range of additional effect potentially achievable will be drawn from and will be equivalent to $E_{max} - E_1$.

The increment of intensity producing a noticeable effect beyond that due to I_1 is ΔI. It corresponds in quantitative properties to the "absolute threshold" ΔI_0 when $I_1 = 0$; the only difference is that with I_1 finite the test starts from a certain level of light adaptation and I_1 is (usually) still present for comparison. Among important properties in which the essential similarity of mean values of ΔI and ΔI_0 are manifest is the rectilinear proportionality to their indices of dispersion in repeated tests,[10] the homologous types of dependence on retinal area[5] and on exposure time,[6] and on the level of light adaptation.[6] Analysis demonstrates that $1/\Delta I_0$, like effect E, gives a normal probability summation as a function of exposure time.[6] In a similar way, the "differential excitability" $1/\Delta I$ is regarded as determined by the summation of neural effects from that part of the total potential population not already excited by I_1. In other words, the capacity to be excited, which is measured by $1/\Delta I$, is determined by (and thus measurable in terms of) the remaining number of unexcited elements. If effect E is a probability integral in $log\ I$, then $1/\Delta I$ must be a similar probability integral with reversed slope, since at each I_1 we will have $1/\Delta I$ equivalent to $E_{max} - E_1$.

It will be noticed that an essential part of this argument involves simply the *number* of elements of neural effect ($dE/d\ log\ I$) and makes no reference to any fixity of the individual unit thresholds in terms of $log\ I$; in the deduction[6] of the probability summation for these cases it is explicitly presumed that the thresholds of these units fluctuate at random. As a matter of fact, the relation[10] between ΔI and $\sigma_{\Delta I}$ permits another method[11] of obtaining the form of the ΔI function, which emphasizes the significance of fluctuating variation of performance in the neural units concerned in statistically determining the nature of the law for sensory effect as a function of intensity; its results are essentially the same as those about to be described.

The tests of this formulation must ultimately involve a good deal more than its ability to apparently describe the data, particularly since customary criteria of curve fitting are of very doubtful utility for such data. Valid tests must be particularly directed toward examining the properties of the parameters of the function. Among the most interesting of such tests are those theoretically possible by altering the organism's temperature. But certain other tests, although in some respects less decisive, can readily be applied. The derivation of the probability summation for

$1/\Delta I$ makes no reference to anatomical specificities of receptor or central nervous organization; it makes no appeal to the photochemical (or perhaps photoelectric) basis of the primary excitation of individual visual cells; it is concerned solely with the fact that the determination of visual response is brought about through the activation of groups of neural units which fluctuate in performance and in accommodation. It should therefore be basically applicable to the data from a great variety of animals. More-

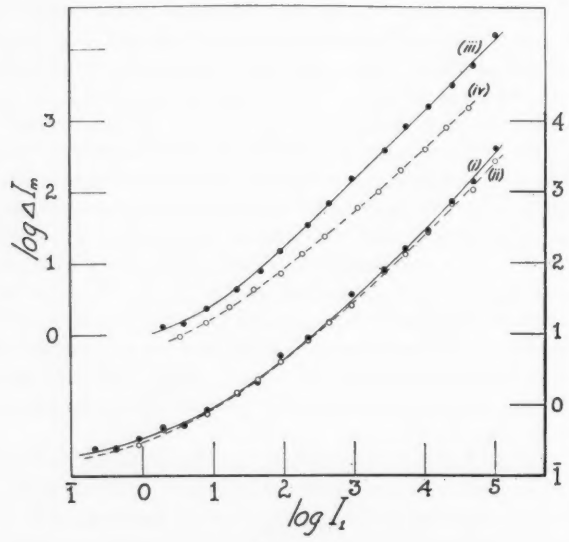

FIGURE 3

Data for small (foveal) fields, from Steinhardt:[16] (i), 56′ test field, no surround; (ii), with an illuminated surround, the effect of the surround is to lower the theoretical minimum log ΔI_0 from $\bar{1}.21$ to $\bar{1}.10$; (iii) and (iv), 23.5′ field with and without surround (illumination relatively greater than with (ii); with (iii) the minimum log $(\Delta I_0)_m = \bar{1}.55$, for (iv), $\bar{1}.45$. The curves are computed probability integrals for $1/\Delta I_m$ as a function of log I_1.

over, it should hold for "instantaneous thresholds" immediately after the removal of an adapting intensity,[12] for ΔI as obtained by the method of increasing I from I_1 to I_2 (no contrast involved, as the illuminated field is uniform), and for the conventional ΔI experiment in which I_1 is continuously present. The experiments of these three types form a graded series as to the operational rôle of I_1, which serves to emphasize the essential nature of ΔI as a threshold intensity under different sets of conditions.

Further, the basic rule should in principle be equally applicable for auditory and other sensory modalities. Mechanical complications due to the existence of several sets of populations of overlapping neural effects may require an extended analysis of such data. Here, only illustrative cases need be given.

Figure 1 is a plot of "instantaneous visual threshold" data from Blanchard.[12] These have been analyzed by Hecht[13] and by Federov[14] from the

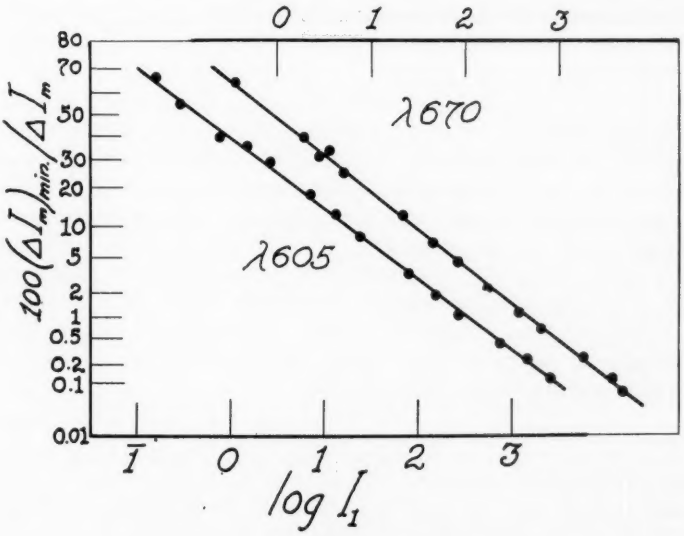

FIGURE 4

Two series of determinations (from Hecht, Peskin and Patt[17]) with red lights (one observer), recalculated to give $1/\Delta I_m$ as a function of $\log I_1$ for the "cone" segment (cf. Fig. 2, etc.), here shown on a probability grid. For λ 605 $\log \Delta I_m = \bar{1}.52$. For λ 670 $\log \Delta I_m = \bar{1}.60$.

(These data actually include several points at lower levels of I_1 which "break" into the "rod" segment. It is to be remarked that when $\Delta I/I_1$ is studied as a function of I_1 the breaking of the whole range of the measurements into two segments is easily overlooked; this has sometimes occasioned the unwarranted suggestion of aberrant qualities in such data.)

photochemical standpoint, on the basis that the threshold effect is a photochemical constant. Figure 2 contains data from experiments by Crozier and Holway[15] using the method of increasing I_1 to I_2 at a constant relative rate. Figure 3 (from measurements by Steinhardt[16]) and figure 4 (from data by Hecht[17]) are from experiments involving the use of I_1 and $\tilde{I}_2$ in comparison fields.

The probability integral in $log\ I_1$ efficiently describes these data, which illustrate the three main types of differential threshold measurements already mentioned. Advantages of dealing with ΔI_m directly, rather than with $\Delta I/I$, are both theoretical and practical. It results that certain subsidiary questions are necessarily viewed in a new light. Thus the much discussed matter of the rise of $\Delta I/I$ at high intensities, and the effect of a "surround" upon this, ceases to have meaning. The presence of a surround enlarges the comparison population of effects (and thus produces the expected changes in the three parameters of the $1/\Delta I - log\ I_1$ integral), but merely pushes the terminal rise of the Weber fraction to higher levels of I_1 (*cf.*[18]).

Summary.—The differential threshold for the visual discrimination of intensities follows the same essential rules as are obeyed by the so-called "absolute" intensity threshold. When $\Delta\bar{I}$ involves the marginal recognition of an increase of intensity above an adapted level, $1/\Delta\bar{I}$ is a declining normal probability integral as a function of $log\ I_1$. This is deduced on the assumption that at any given level of visual accommodation the excitability measured by $1/\Delta I$ is determined by the not-already-excited portion of the total population of potentially excitable neural effects. There is obtained in this way, without unnecessary assumptions, a simple, efficient formulation of the existing data which is consistent with the statistical basis of neural discriminations in general.

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[6] Crozier, W. J., *Ibid.*, **22**, 412 (1936); *Ibid.*, **26**, 54 (1940*a*); *Ibid.*, **26** (in press) (1940*b*).

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[9] Cf.[7,8].

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DIFFERENCES BETWEEN MEN AND WOMEN IN THEIR RESPONSE TO HEAT AND COLD

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Read before the Academy, April 22, 1940

There have been a large number of publications dealing with the reactions of men to changes of environmental temperature, but very few studies with women. Just why there has been such a neglect of half our population is not clear. Air conditioning is theoretically based on physiological studies, and politeness alone would demand more consideration of the ladies. It is well known that women in light clothing tolerate a wide range in temperature. This is not due to greater fortitude or vanity but rather a better physiological adjustment.

Max Rubner in 1890 and 1902[1–3] outlined the factors concerned in the loss of heat and described the "physical regulation" through changes in heat loss or voluntary activity. He also spoke of a rise in heat production through "chemical regulation" which is induced without visible changes in the activity of the animal. In previous papers from the Russell Sage Institute of Pathology[4,5] presented before this Society we reported that the heat production of the two normal men studied by us in detail was uniform when exposed to temperatures between 22° and 35°C. This is in accordance with the findings of Wiley and Newburgh[6] in Ann Arbor, Winslow, Herrington and Gagge[7] in New Haven, and others. Martin,[8] however, did note a falling metabolism in one man during a voyage to the tropics. There have been may reports indicating that the basal metabolism of men and women is lower in the tropics, but there are several factors involved besides temperature. Miss Mason,[9] who eliminated all the factors except climate, observed that the metabolism of a group of women was 5% lower in tropical India than it was in the temperate zone. Hardy and Milhorat,[10] in a brief preliminary publication from the Russell Sage Institute of Pathology last year, demonstrated that three normal women showed a considerable drop in metabolism in a warm environment and

they analyzed the differences between these women and the two men that we had studied in the same manner. In this present report we can add the results on four more women.

The men and women were studied in the respiration calorimeter of the Russell Sage Institute of Pathology[11] which is now at the New York Hospital. They came to the laboratory at 9 o'clock in the morning without breakfast, sat quietly for an hour in the calorimeter room which had been kept at the desired temperature since the previous day, undressed and entered the calorimeter which was then sealed. During the preliminary period in the box the surface temperature was measured. The first experimental period was started at about 11 o'clock, and observations were terminated either at noon or at 1 o'clock. At the end of the experiment the surface temperature was determined once more. During the experimental periods the subjects lay almost motionless on a comfortable bed made of fish net with a folded sheet under the body. There was little movement of air in the calorimeter. The two normal men were 33 and 54 years old, in good physical condition; and the seven women were artist models or technicians, in good physical condition, between the ages of 21 and 35 years, with weights of 54 kg. to 77 kg. They were all intelligent and coöperative.

The respiration calorimeter of the Russell Sage Institute of Pathology has been described many times. Since 1935 we have been using the Hardy radiometer[12] to determine skin temperature. Combining these two instruments the following quantities are measured:

(1) The rectal temperature, measured every 4 minutes
(2) The skin temperature in twenty points, at the beginning and end of each period
(3) The calorimeter temperature (wall and air), at the beginning and end of each period
(4) The total heat production during a period (usually one hour)
(5) The total heat loss by radiation and convection
(6) The total grams of water vaporized from skin and lungs
(7) The surface area of the body

From these data, according to procedures already described in detail, the following quantities could be calculated:

(1) The average skin temperature
(2) The effective radiating surface of the skin
(3) The total heat loss by radiation
(4) The total heat loss by convection
(5) The total heat loss by vaporization

(6) The heat stored in the body
(7) The conductance of the peripheral tissues of the body
(8) The cooling constant of Newton's Law of Cooling

Figure 1, which has already been described in previous publications[4,5] shows the results on the two men. It will be noted that the heat production represented by the first blank column in each group of experiments

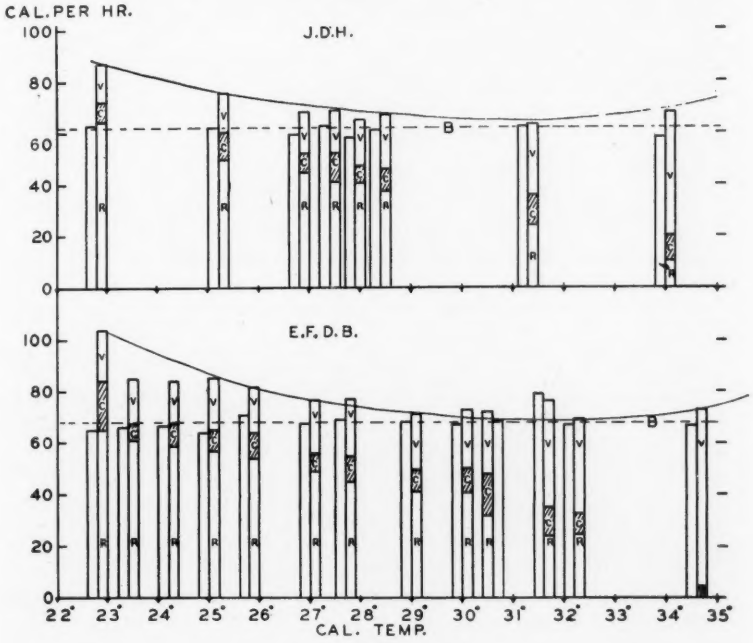

FIGURE 1

Heat production and heat loss of two normal men exposed naked in the calorimeter to temperatures between 22–35°C. The first or blank column in each experiment shows the basal heat production (*B*) which was uniform throughout the range. The second column shows heat loss divided into radiation (*R*), convection (*C*) and vaporization (*V*).

remained constant throughout the experimental range. The heat loss shown in the second column of each group exceeded the heat production in the cold zone and equalled it in the comfort zone between 29° and 31°C. Radiation (*R*), given in the lower portions of each of these heat loss columns, decreased steadily with increasing temperature until it disappeared at 35°C. when the skin temperature equalled the temperature of the wall of

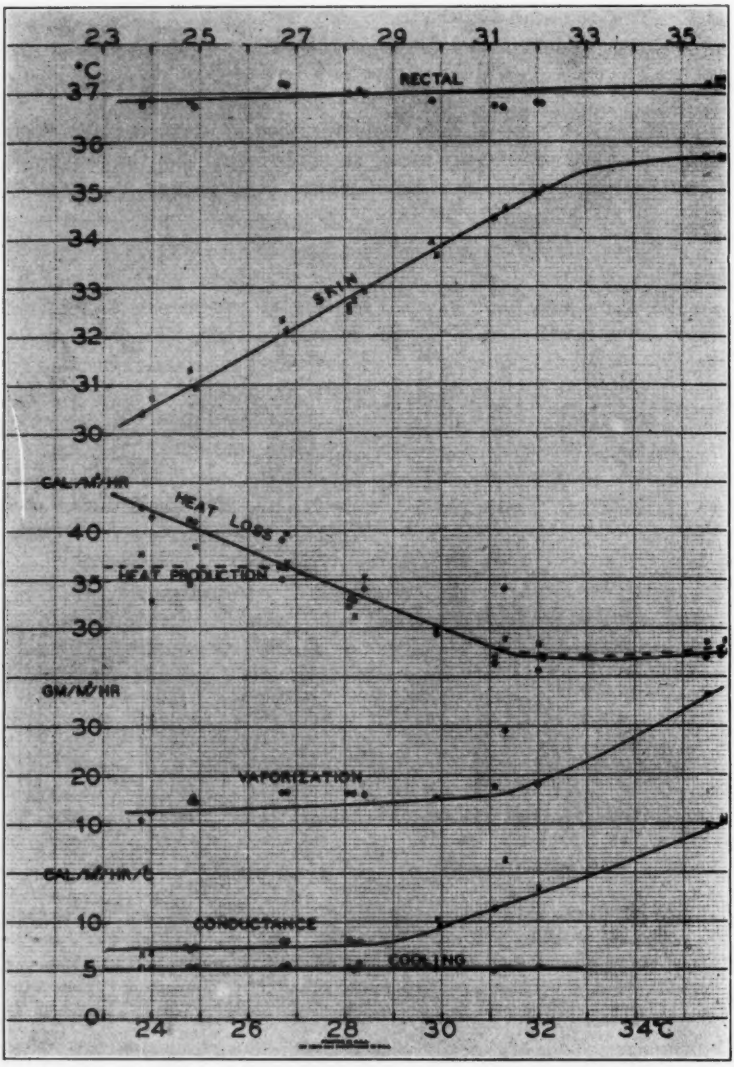

FIGURE 2

(Figures 2 and 3.) Calorimeter data obtained in two normal women showing the fall in heat production in temperatures above 27°C.

the calorimeter. Convection (C) played a relatively small rôle because there was slight air movement in the calorimeter and loss by convection

disappeared when the air temperature was the same as the skin temperature. Vaporization (V) remained fairly uniform until the air temperature rose above 30°C., then increased rapidly until it assumed the whole burden of heat loss at 35°C.

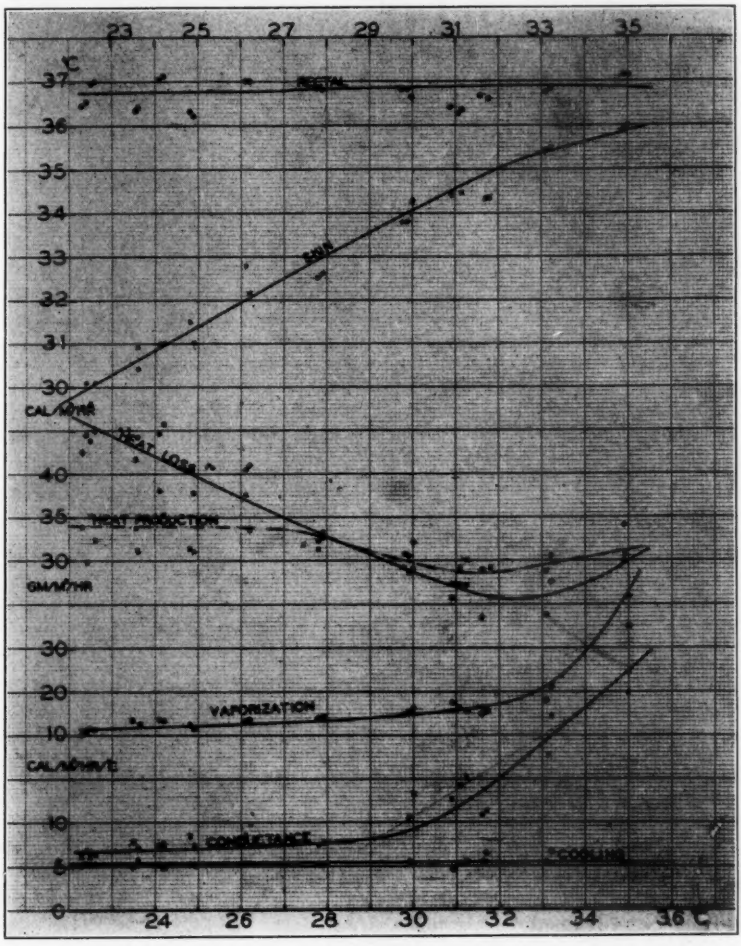

FIGURE 3

The results on three of the young women are shown in figures 2, 3 and 4. The first two demonstrate a marked fall in heat production in the warm zone. The third woman was an exception, for there was practically no

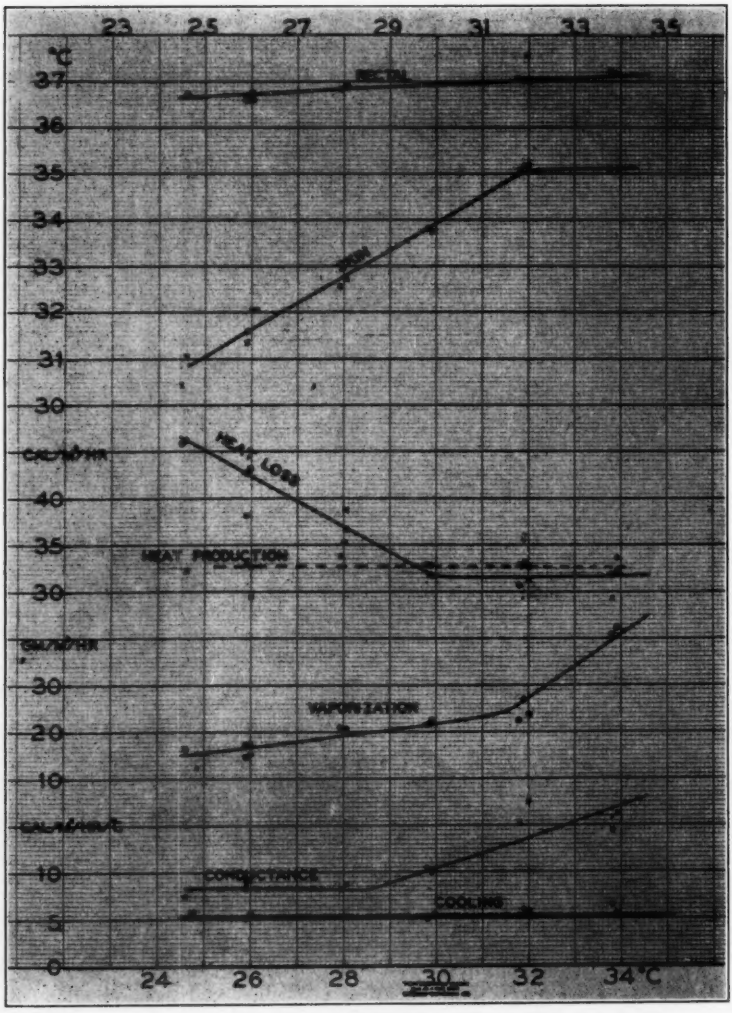

FIGURE 4

The one woman in a series of seven who showed no fall in heat production with rising temperatures.

change in heat production between air temperatures of 24.5° and 34°C. It so happens that this woman was being treated with hormones for amenorrhea. Whether or not this is significant we do not know. Figure 5

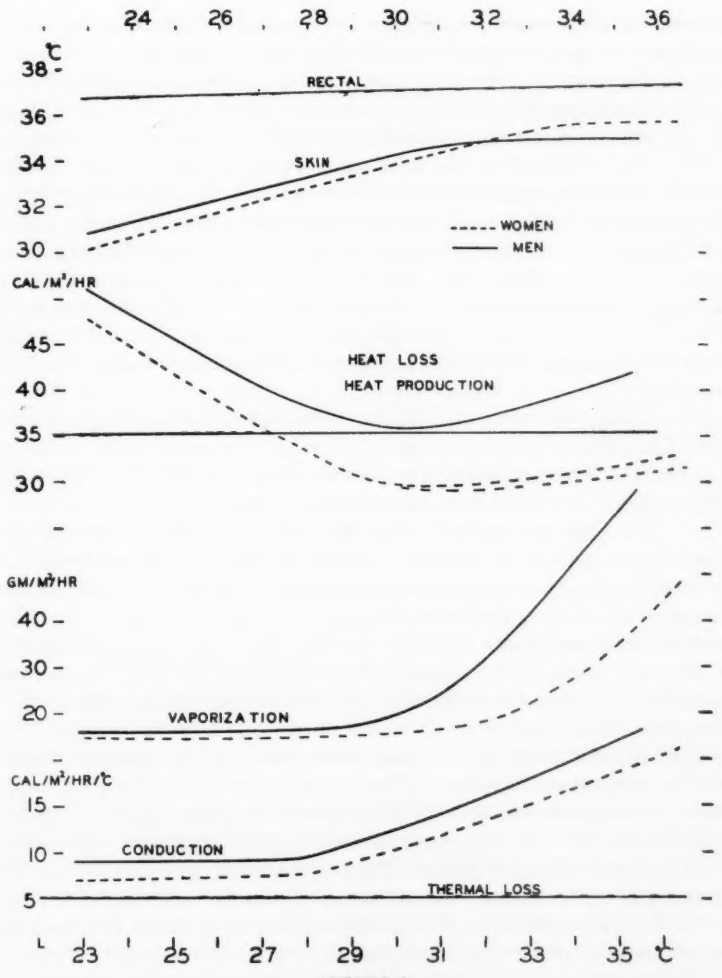

FIGURE 5

A comparison of results obtained on men and women at calorimeter temperatures between 22–35°C. Note that the dotted line showing the heat production of the women is almost exactly the same as that of the men in temperatures between 23–27°C. and then falls, almost equaling the upper dotted line which shows the heat loss in women.

shows the differences between the results obtained on the men and women. The measurements made after about $2^1/_2$ hours' exposure, naked, and motionless, to the various temperatures, are plotted according to the

calorimeter temperature in degrees Centigrade. Needless to say it required many days to complete the tests on any one subject.

(1) The rectal temperatures were the same for the men and women and were both slightly lower in the cold zone as a result of exposure to the cold air. There was a spread of about 0.6°C. in the individual measurements.

(2) After exposure to the cold air for approximately $2^1/_2$ hours the average skin temperature of women was about 1.0°C. lower than that of the men. In the warmest zone skin temperatures after the same exposure were about 1.7°C. higher than those of the men. In environments colder than 33°C. the average skin temperature fell almost linearly with the calorimeter temperature so that a change of 1° in the calorimeter caused a change of 0.5° in the skin. The change in the women was greater than that observed in the men by 0.1°C. per degree fall in the environmental temperature.

(3) The heat loss for women in the cold zone was about 10% lower than that of the men on account of the lower skin temperature. In the warm zone the heat loss of most of the women was about 14–20% lower than that of the men because the women did not sweat as much.

(4) The heat production of the men was constant throughout the temperature range. In marked contrast to this the heat production of the women which was the same as that of the men in the cold zone showed a marked fall in temperatures about 27°C. Above this point the heat production of the women fell with the heat loss being almost equal to it at all times. Therefore, in temperatures from 30° to 32°C. under our standard conditions the metabolism of most of the women was 14–20% lower than that of the men.

(5) Vaporization in the cold zone is accounted for by the loss of water from the respiratory passages and by seepage of moisture through the skin. There is no appreciable activity of the sweat glands in the cold. In this zone the vaporization was approximately equal in men and women, but as the temperatures grew warmer the men started to sweat at 29°C. The women did not start until the air temperature had been raised to about 32–33°C. They did not need to sweat as soon or as much because they had lowered the production of heat in their bodies. At temperatures around 34–36°C. both sexes lost all their heat through vaporization because the skin temperature had equaled the air and wall temperatures and radiation and convection had disappeared as channels of heat loss.

(6) Conductance, (conduction, conductivity) represents the ease with which heat is transferred from the interior of the body at a temperature of 37°C. to the surface, which is at a much lower temperature. Conductance can be calculated from the total heat lost in hourly periods knowing the rectal temperature and the average skin temperature. Under all conditions heat is transferred by conduction through the skin and subcu-

taneous tissues, which are about as good insulators as cork. In the cold these tissues are almost bloodless. The lower conductance for women in the cold is due to the greater thickness of the subcutaneous tissues, and we calculate that the average difference represents a layer of fat about 4 mm. thick. We have found that fat has about the same conductance as muscle, skin or cork.

The upward swing of the curves in both sexes which begins at 28°C. is due to a new factor, the steadily increasing transport of heat by the blood stream. With rising environmental temperatures there is an increased vaso-dilatation. Although the hands and feet show the most dramatic changes in blood flow they play a relatively insignificant part since they represent only 12% of the total body surface.

(7) As an over-all check on the physical procedure and measurements we have calculated the thermal loss or constant for Newton's Law of Cooling. This represents the heat loss by radiation plus convection per unit area of skin per degree of difference between skin and air temperatures. As would be expected the constant is the same for the two sexes and does not change with calorimeter temperature. For radiation alone calculation with the Stephan-Boltzman Law gives much closer results.

In basal metabolism tests with men and women little attention has been paid to the environmental temperature as long as the patient was comfortable. It is, of course, difficult to estimate or measure the actual environmental temperature of a person who is clothed or covered with a blanket. We have not yet made determinations on clothed subjects, and it must be remembered that we have made our measurements only on persons at complete rest, naked and in an environment with minimal air movement. The results, however, indicate that it may be necessary to make changes in the standards of the normal basal metabolism, paying attention to the environmental temperature in the case of women. The lowering of the metabolism in the women in the warm zone speaks for the existence of a "chemical regulation" as described by Rubner for animals. This had never previously been established for the human species although it is generally accepted for animals. The physical regulation of temperature involves changes in the skin, sweating and other physical methods of heat loss and the changes in heat production due to exercise or unconscious tensing of the muscles. The chemical regulation is supposed to be due to some non-physical factor such as a hormone, which changes the level of heat production. The prevailing opinion is that such regulation would appear as the environment began to grow colder and would increase metabolism. Our calorimeter data favor the idea of a factor which lowers the metabolism in warm atmospheres from the standard level established in a cooler zone.

Summary.—The men and women whom we have studied under our limited standard conditions showed two points of agreement—Newton's

Law constant and the internal body temperature. In all the other adjustments to changes in the thermal environment the women have a physiological advantage. The comfort zone, in which the heat loss and heat production are equal, extends over a range of about 6° for women instead of 2–3° for men. The most important factor is the fall in heat production of women in warmer environments. Another factor is the thicker layer of insulation against cold. A third factor is a slightly better adaptation of skin temperature to meet thermal changes in the environment. In cool air women, lightly clad, may be comfortable when the men need woolen clothing. In the warm zone long before the women have started to perspire or even "glow" the men may be covered with beads of sweat. Many of these facts have been suspected for generations but all should be taken into consideration in the practice of air conditioning.

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ON POSSIBLE CHANGES IN THE SOLAR "CONSTANT"

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Communicated May 8, 1940

1. Introduction.—In an earlier communication[1] (here called "paper I"), ten periodicities suggested[2] by Abbot were tested for statistical significance, in order to see whether any evidence could be obtained, from measures of the solar constant, of the possible existence of changes in the solar radiation. It was found that three harmonic components (with periods of 7, 8 and 34 months) could be attributed entirely to the effects of ordinary random errors. The other seven (of $9\frac{3}{4}$, 11, 21, 25, $39\frac{1}{2}$, 46 and 68 months) could not, and therefore they arose either from changes in the solar radiation, or from systematic errors. There was, accordingly, something that it might be profitable to study further.

We describe in the present paper a thorough periodogram analysis of the solar constant, made without regard to Abbot's suggested periods, and with no assumptions about the lengths of any possible periods. We find ten pronounced harmonic components, statistically significant in the sense of their not arising from random errors, but not necessarily real in the sense of corresponding to changes in the solar radiation. Their periods do not correspond in detail to Abbot's. They must exist either (*a*) in the true values of the solar constant, or else (*b*) in the systematic errors. It is not definitely determinable from which of the two sources, (*a*) or (*b*), they come. Yet the six longest periods, among the ten that are found, are submultiples of 10.2 years and correspond to terms in the Fourier expansion of a variation fundamentally periodic in a 10.2-year period. The average length of the two sun-spot cycles that most closely overlap the 15-year interval of observation is very nearly 10.0 years. The two lengths, 10.2 and 10.0, are equal within the uncertainties of their determination. Therefore, either a fundamental periodicity (not necessarily permanent, but still pronounced over an interval of 15 years), equal in length to the sun-spot period, has somehow crept into the systematic errors, or else there are changes in the amount of the solar radiation. The latter changes must have been periodic during an interval of fifteen years, but they do not have to be of a permanently continuing sort. In this paper we are not interested in the permanency of periodicities. We make use of harmonic analysis, but only in order to see whether we can find evidence, by doing so, for the occurrence of any real changes at all in the amount of the solar radiation.

2. The Analysis.—If the observed values of the solar constant contain

any periodically varying part, then that part must be composed of sinusoidal components whose periods are integral submultiples of the period of the variation. Mathematically, the situation is simple. Each periodicity in the values of the solar constant can be represented by a Fourier series, periodic in the period, $2\pi/s$, of the periodicity. Besides a trivial constant term, the Fourier series consists of pairs (harmonic components) of the form $b \sin nst + c \cos nst$ where t is the time and n is a positive integer, not zero. It is easy to find, by least squares, the values of b and c for a harmonic component having any specified period. This was done, for periods specified by Abbot, in paper I. Abbot does not appear to have chosen his periods through any systematic periodogram analysis, but rather through a process of inspection and trial. We have therefore subjected the material that was examined in paper I to a thorough periodogram analysis of a modified Schuster type. The material consists of 540 ten-day mean values (strictly, "decade" means, over intervals of one thirty-sixth of a year) of the solar constant, covering the interval from 1920 through 1934, and published by Abbot[3] from measures made by the Smithsonian Astrophysical Observatory.

The analysis was carried out for each of 76 trial periods, P_u, having reciprocals, v_u, given by the formula

$$v_u = 0.0048 + 0.000926\, u \qquad (1)$$

in periods per decade, where $u = -1, 0, 1, 2, 3, \ldots . 74$. The longest trial period was thus about 86 months; it was considered unsafe to use longer trial periods with material covering only 180 months. Equation (1) yields a set of trial frequencies spaced at one-half the resolving power. The shortest trial period was 4.55 months, since the number of different trial frequencies that could conveniently be employed was limited by the eighty columns of the punched-card computing machinery. The observations were numbered serially from 1 through 540. The phase (expressed as a fraction of the trial period) of the Nth observation, according to the trial frequency v_u, was thus the decimal part of Nv_u. For each trial period, the observations were classified into ten phase-classes, with phases (rounded to the nearest tenth) running from 0.0 through 0.9. The means, M_j, of the values of the solar constant in the respective phase-classes were then used as the right-hand members of observational equations of the form

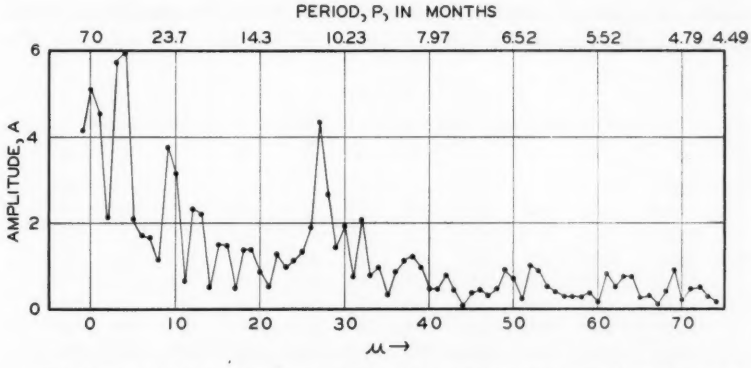
$$a + b \sin 36°j + c \cos 36°j = M_j; \qquad j = 0,1,2 \ldots 9. \qquad (2)$$

There were thus ten observational equations for each trial period. The ten equations were assigned equal weights (A) in a least squares solution for the coefficients a, b and c.

It would have been slightly more accurate to have adopted, for the weights (B) of the equations, within each least squares solution, the popu-

lations of the respective phase-classes. The populations in question, however, were all close to 54 and it is known, in general, that least squares values are fairly insensitive to changes in the weights. The adopted system of weights (A) therefore leads to almost exactly the same values of the coefficients as would the system (B), while it facilitates the computations by reducing, to a single form, all of the different least squares solutions.

The results of the preceding analysis are shown in table 1, and in the diagram. Values of the amplitude, $A = (b^2 + c^2)^{1/2}$, are listed in the table against the corresponding values of u, and are plotted against u in the diagram, which constitutes a periodogram. As it relates to u, the resolving power is constant and equal to two units throughout the diagram; as it relates to P_u, the resolving power varies. The straight lines joining the



Periodogram of the ten-day mean values of the solar constant, covering 15 years of observation. The amplitude is in units of 10^{-3} calories per square centimeter per minute.

computed points have been drawn merely to assist the eye, and have no other significance.

Corresponding to any harmonic term having a frequency v', in the observed values of the solar constant, there exists a certain pattern in the periodogram. It is easy to show* that the pattern consists of a principal maximum or "peak" at $v = v'$, flanked by secondary maxima located very nearly at $v' \pm 3h/2, 5h/2, 7h/2$, etc. The ordinate falls to minimum values between them, at $v' \pm h, 2h, 3h, \ldots$ etc. The quantity h, the resolving power, is the reciprocal of the interval of observation, and is here equal to 0.001852 decades^{-1}. The heights of the principal and secondary maxima are very nearly in the ratio of $1:(2/3\pi):(2/5\pi):(2/7\pi):\ldots$, etc. The pattern is similar to one produced by a diffraction grating having a small number of lines. The occurrence of random errors in the material of course

tends to mask all the peaks to some extent, and the secondary maxima, being smaller than the principal maximum, are the more easily obscured.

It will be noticed that in accordance with equation (1), a change of unity in u changes the trial frequency v by an amount equal to one-half the resolving power. This spacing was adopted in order to cover the periodogram thoroughly, over the interval tested. With this spacing, each separate principal peak is covered by three or more points.

3. Results.—The periodogram contains a considerable number of peaks. In order not to be distracted by the smallest ones, most of which are probably mere statistical fluctuations, let one consider only the twelve maxima on which one or more of the computed ordinates exceed 10^{-3} calories per square centimeter per minute. By interpolation between the computed ordinates, which correspond to integral values of u, the fractional values of u may be estimated that correspond to the maxima of these peaks. Such interpolated maxima probably yield the best values of the

TABLE 1

ORDINATES OF THE PERIODOGRAM IN UNITS OF 10^{-5} CAL./CM.^{2} MIN.

u	0	1	2	3	4	5	6	7	8	9
−10	...	...	...	...	...	...	...	...	...	418
0	508	457	213	532	594	211	173	164	115	376
+10	316	064	233	221	050	151	145	047	136	136
20	084	052	126	098	113	130	192	436	264	142
30	194	075	206	078	096	035	085	112	121	096
40	047	046	078	043	006	039	042	031	046	088
50	073	023	100	087	054	040	027	028	026	039
60	017	081	052	075	078	026	030	009	042	091
70	020	045	052	026	015	...	...	...	...	...

periods that can be found without the considerable labor of computing additional points on the periodogram, and they are listed in table 2, in the first column. The second column contains the corresponding frequency, v, in periods per decade; the third, the period in months; and the last column, the value of P_{bc} obtained as in paper I. The number P_{bc} is the probability, approximately, that statistical fluctuations in the random† errors would give rise to a value of $t_b^2 + t_c^2$ equal to or larger than the value observed for the greatest computed ordinate in the peak. Here t_b and t_c are the ratios of b and c to their (equal) mean errors, and thus the value of P_{bc} depends both upon A and upon the residuals of the observational equations. It is difficult to estimate, accurately, the uncertainty in the interpolated u's. If one supposes that such u's have uncertainties of the order of 0.5, then the frequencies have uncertainties of the order of 0.0005. The last two digits of the periods in column three are probably doubtful.

The resolving power is two units in u; and if one supposes that there are thirty-eight independent trial periods between $u = -1$ and $u = 74$, then one should expect to find only one or two peaks, within this range, with P_{bc}'s that are equal to or smaller than 0.03, and that arise by chance from the effects of random errors. The expected number is if anything fewer than two, and probably all but two or three of the above peaks must be considered to have statistical significance.

The distribution and heights of the first six peaks in table 2, which appear with the small doubtful seventh peak to form a related family in the periodogram, do not allow the family to be readily explained as being due to the diffraction patterns of a few "principal" peaks. The first six peaks appear to have periods that are closely equal to submultiples of a fundamental period of 368 decades, or 10.2 years. With this fundamental period, a periodicity would have for the u's of its 2nd, 3rd, 5th, 6th, 7th and 8th

TABLE 2

u	ν (PER DECADE)	P (MONTHS)	P_{bc}
0.14	0.00493	67.6	7×10^{-4}
3.64	0.00817	40.8	4×10^{-6}
9.31	0.01342	24.8	1×10^{-40}
12.43	0.01631	20.4	3×10^{-3}
15.44	0.01910	17.45	1×10^{-2}
18.50	0.02193	15.20	4×10^{-3}
22.23	0.02538	13.13	3×10^{-2}
27.09	0.02989	11.15	9×10^{-25}
29.80	0.03239	10.29	7×10^{-6}
32.01	0.03444	9.68	3×10^{-5}
37.76	0.03977	8.38	2×10^{-2}
52.36	0.05329	6.26	3×10^{-3}

harmonic Fourier components[‡] the values 0.69, 3.62, 9.50, 12.43, 15.37 and 18.30, in close agreement with the observed values. The value 368 decades was obtained by least squares from the first six peaks. The peak at 13.1 months does not fit into this scheme, but it is low, its P_{bc} is the largest in table 2, and it is of no statistical or physical significance. The peak at 17.4 months, although it fits into the scheme, has a P_{bc} of 0.01, a value hardly small enough to be of statistical significance among so many trial periods. This value of P_{bc}, however, corresponds to the integer $u = 15$; and it appears from the periodogram that if the ordinate at 15.44 were exactly computed, its P_{bc} would be considerably smaller. It is therefore considered that this peak is probably statistically significant.

The eleventh Fourier component of a fundamental period of 368 decades would have a u of 27.11, sufficiently close to the high peak observed at $u = 27.09$ (11.2 months); but to find so large an amplitude for so high a harmonic would not be likely, and it may not be reasonable to attempt to

relate this large peak to the fundamental period of 10.2 years. The peaks at 10.3 and 9.7 months may be the first and second subsidiary maxima associated with the principal maximum at 11.2 months. The spacing is right for this interpretation, and the plotted heights, it should be remembered, are not the true heights since our analysis has been carried out only for integral values of u. The small peak at 8.4 months does not appear to have even statistical significance; that at 6.3 months appears to be statistically significant but it may not be related to any of the other peaks in table 2.

The minimum to the left of the peak at 11.2 months appears at $u = 23$ instead of at $u = 25.09$ where it ought to be, two units to the left of the maximum. There thus appears to be some evidence, from the periodogram, for the existence of an extra period somewhat longer than 11.2 months. A period of one year would have a peak at $u = 24.81$, nearly at the first minimum of the pattern associated with an 11.2-month period. A significant P_{bc} was obtained for this annual periodicity in paper I. Perhaps an annual period (a systematic error arising from the atmosphere) contributes to the periodogram a peak that merges with the diffraction pattern of the 11.2-month period to produce the observed peak as a resultant; if this is true then the interpolated value of 11.2 months may be slightly in error.

To sum up, there appears to be a family of statistically significant harmonic components with periods of about 68, 41, 25, 20, 17.4 and 15.2 months. These periods are equal to submultiples of 123 months, closely enough for it to be possible to regard them as the Fourier components of a fundamental period of 123 months. There are also significant harmonic components which, whether related to the preceding periods or not, have periods of about 11.2 and 6.3 months. Significant periods, indicated at 10.3 and 9.7 months, may possibly be "diffraction" phenomena associated with the pronounced period at 11.2 months. There is some evidence for a possible annual term. The present list of periods differs from Abbot's. His 68-month period is the same as ours, but his 46-month period falls upon the rising portion of the 41-month peak and does not appear to have a separate existence. His $39^1/_2$-month period appears in our list as 41 months. His 34-month period corresponds to a minimum in the periodogram and does not seem to exist. His 25-month period is in our list, and his 21-month period is our 20-month period. Our 17.4 and 15.2-month periods are absent from his list; his 11-month period is our 11.2-month period; our 10.3-month period is missing from his list; and his 9.75-month period is our 9.7-month period. His 7 and 8-month periods come close to minima and do not appear to exist, while our 6.3-month period is missing from his list.

That six of the ten components should appear to be overtones of a funda-

mental periodicity of about 10.2 years suggests, to some extent, a dependence of the solar radiation itself upon the phenomena that underlie the sun-spot, and other solar periods. Over the 15-year interval during which the observations of the solar constant were obtained, the average length of two sun-spot cycles appears to have been about 10.0 years. As pointed out in the *Introduction*, this implies that unless a fundamental period equal to the sun-spot period has somehow crept into the systematic errors then there must be changes in the true solar constant itself.

Dr. Abbot has kindly informed us that he plans to publish, shortly, values of the solar constant from 1920 through 1939—values computed by improved methods of reduction. The new systematic errors may be smaller, and in any case should be different. It is planned to obtain a periodogram of the new material when it is published. A comparison of that periodogram with the present one may possibly make more certain the origin of the periodicities—whether they come from the systematic errors, or from real changes in the solar radiation. Until then, we wish to suspend further judgment on this most important question.

The authors wish to thank the Harvard Graduate School of Business Administration, and in particular Professor Theodore H. Brown, for very kindly allowing us to use their punched-card computing machinery, of the Hollerith type.

4. Summary.—A thorough periodogram analysis has been made of five hundred and forty ten-day mean values of the solar constant, published by Abbot. Seventy-six equally spaced trial frequencies have been used, corresponding to periods between 86 and 4.6 months. The analyzed material contains harmonic components (statistically significant in the sense of not arising from random errors, but not necessarily real in the sense of corresponding to changes in the solar constant), with amplitudes greater than 10^{-3} cal./cm.^{2} min., at 68, 41, 25, 20, 17.4, 15.2, 11.2 and 6.3 months. The last digits are uncertain. Two other peaks, at 10.3 and 9.7 months, may perhaps be subsidiary maxima associated with the 11.2-month component. The new list of periods differs in detail from Abbot's, and the six longest members appear to be the Fourier components of a fundamental periodicity having a length of 10.2 years. Within the accuracy of their determinations, this last period, and the average sun-spot period at the time of the observations, appear to be equal. The equality suggests the occurrence of some real changes in the solar constant, but pending the publication by Abbot of revised material, this point should not be regarded as settled.

* . . . by considering, analytically, the nature of the periodogram that would be obtained from a quantity $a + b \sin 2\pi v'N + c \cos 2\pi v'N$.

† The measures of the solar constant may be considered to consist of the solar constant, plus random errors, plus systematic errors. By *random* errors, here and in paper I,

errors are referred to that may be considered to have been drawn independently and at random from some parent population whose mean is zero. Errors of all other sorts are called *systematic*. Hulme,[4] in a short paper with much of which we agree, appears to misunderstand this fairly conventional use of the expression *random errors*. Correlated errors, for us, are systematic errors.

‡ There is, of course, no reason why the 4th component should not be small.

[1] Sterne, *Proc. Nat. Acad. Sci.*, **25**, 559 (1939).

[2] Abbot, *Smithsonian Misc. Coll.*, **94**, No. 10 (1935).

[3] Abbot, *Ibid.*, p. 12.

[4] Hulme, *Observatory*, **63**, 101 (1940).

ON SOLAR FACULAE AND SOLAR CONSTANT VARIATIONS

By Henryk Arctowski

Smithsonian Institution

Read before the Academy, April 23, 1940

On January 4, 1845, experimenting with a thermopile of Ruhmkorff Professors Henry and Alexander of Princeton[1] made "twelve sets of observations, all of which, except one, gave the same indication, namely, that the sunspots emitted less heat than the surrounding parts of the luminous disc."

Exact measurements of the thermal conditions of sun-spots were made by S. P. Langley in 1874 and 1875.[2] He found that "taking the mean thermal photospheric radiation in the spots vicinity as unity, the mean umbral radiation is 0.54 ± 0.005, the mean penumbral 0.85 ± 0.01." Let us notice also that the ratios of the radiation from the umbrae and the neighboring photosphere, observed by W. E. Wilson[3] in 1893 and 1894, varied between 0.29 and 0.85, and that E. B. Frost,[4] comparing Langley's observations, made about three years after a solar maximum, with his own, made about two years after a minimum, writes that "it is impossible to assert that the thermal conditions of spots (and perhaps of the photosphere and atmosphere) are invariable during the eleven-year period of solar activity."

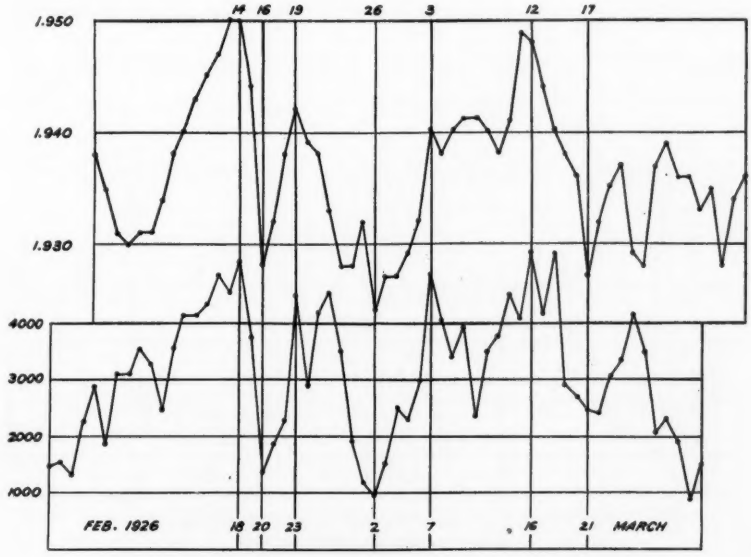
The solar faculae were correctly described long ago by Christ Scheiner in his "Rosa Ursina . . . " published in 1626, and by I. B. Ricciolo, as well, in his "Almagestum Novum . . . " published in 1651.

Secchi noticed that when a facula is observed at the edge of the solar disc, the chromosphere nearby extends higher. J. Fényi,[5] who cites this observation, writes that in the spectrum of faculae the lines of prominences appear very bright, the *K* line in particular. This fact allowed Hale and Deslandres to photograph the distribution of faculae all over the solar disc. Fényi considers the faculae as being redescending gas masses of the ascending prominences. He thinks it is because of adiabatic compression that

the temperature of faculae should be higher than that on the corresponding levels of the solar atmosphere.

If, therefore, the spectrobolometric measurements of C. G. Abbot, F. B. Fowle and L. B. Aldrich, giving the mean radiation along the radius of the solar disc,[6] are accepted as standard, local deviations on the surface of the sun should be admitted, *a priori*, in particular over the areas occupied by faculae.

Daily contrast numbers of brightness, solar center-limb, observed by Dr.



Variations of the solar constant and of areas of solar faculae

FIGURE 1

Daily solar constant values for February and March, 1926, and areas of faculae.

Abbot and his co-workers in 1913 and 1914, compare well, in their variations, with those of solar constant values.[7]

It was these facts which, in 1915, led me to the study of the variations of the daily areas of faculae, and their monthly means,[8] as recorded in the Greenwich photo-heliographic results.[9]

I found that, during the approximately 11-year period of sun-spots, there are five maxima of the quotients of faculae and umbrae areas and that the frequency variations of sun-spots should be considered as a subordinate manifestation of the variations of faculae.[10]

Dr. Abbot's researches on solar constant data led him to the conclusion

that "an increase of 0.07 calory per square centimeter per minute in the solar constant accompanies an increase of 100 sunspot numbers," while my researches on the fluctuations of the solar constant led me to believe that sun-spots should be considered as only one of the acting factors, probably less important than the faculae or other unmentioned solar phenomena on which the areas of faculae depend.[12]

The fact is that F. E. Fowle, comparing the daily data of solar constants of radiation, observed during March, April and May, 1920, with the areas of calcium flocculi, measured at the del Ebro Observatory, noticed an excellent agreement of their variations.[13] Besides, H. H. Clayton ascertained

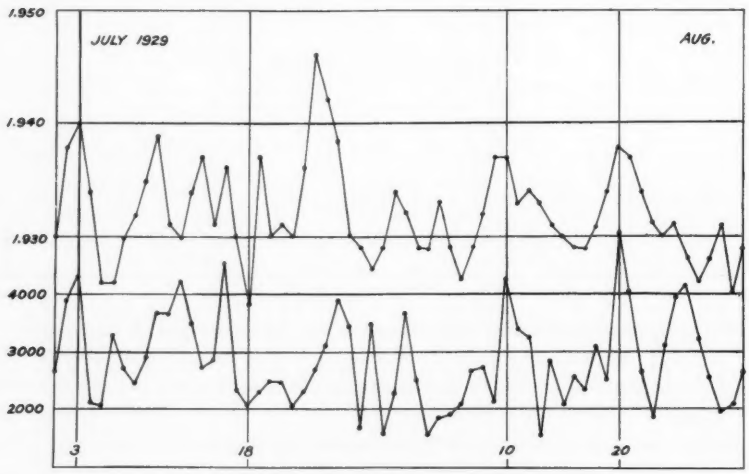


FIGURE 2

Solar constants and faculae, July and August, 1929.

that from July, 1918, to December, 1921, maxima of faculae coincided with maxima of solar radiation, and beginning in May, 1924, he has made forecasts of solar constants from the visual observations of sun-spots and faculae.[14]

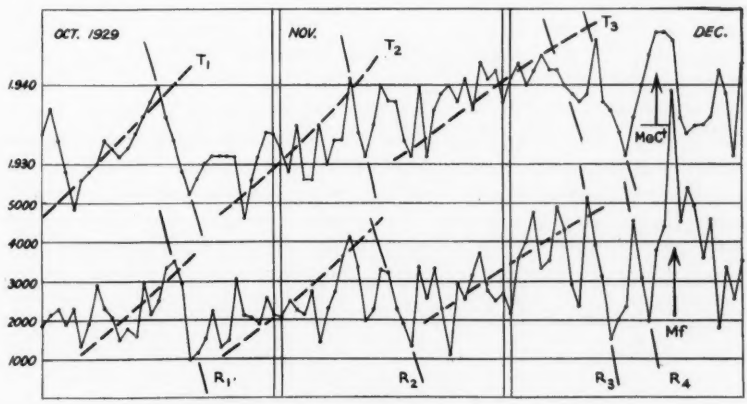
I began my work at the Smithsonian, last December, by studying the daily values of preferred solar constants observed from 1926 to 1930.[15] Dr. Abbot has given the following grades to the tabulated data: S satisfactory, $S-$ not quite satisfactory, U unsatisfactory, $U+$ better than unsatisfactory and $U-$ very unsatisfactory. Four hundred ninety-four of these values, approximately only one-fourth of the total number of observations, were considered perfectly satisfactory. It follows that the es-

tablishment of many solar constant observatories would be advisable if continuous solar constant data are ever to be used in daily weather forecastings, in order to avoid unsatisfactory data which are due exclusively to the temporary local unfavorable atmospheric conditions.

At present let us examine the question of the cause of solar constant variations.

Leaving aside the eventual correlations between sun-spots (areas of umbrae) and the Smithsonian solar constant values, which lead to contradictory results, as I showed long ago, it is only the Greenwich data of areas of faculae, corrected for foreshortening, that will be taken into consideration.

Comparing the diagrams of areas of faculae, expressed in millionths of



Discontinuous trends in solar constant and solar faculae

FIGURE 3

Solar constants and faculae, October, November and December, 1929.

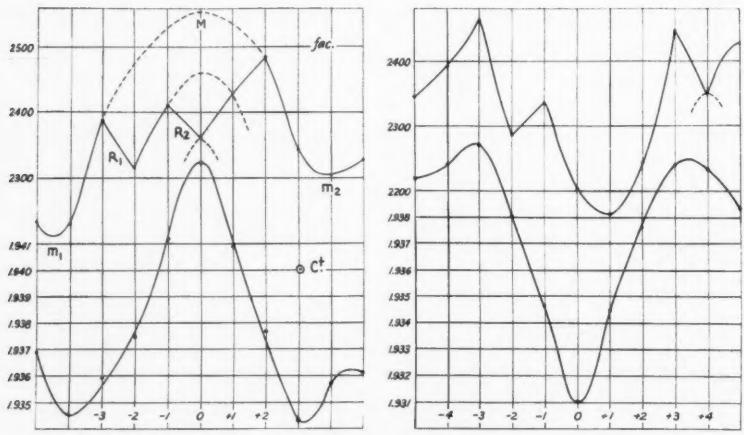
the sun's visible hemisphere, with those of the solar constant values, I noticed that, in order to observe a more or less satisfactory agreement, we must admit that in most cases the maxima and minima of solar radiation precede those of faculae. Taking the daily data of February and March, 1926 (Fig. 1), we must admit a close agreement between the radiation minimum on February 16th and the minimum of faculae on the 20th. But this difference, of four days' advance, is far from being general. Maxima on July 3, August 10 and 20, 1929, for example (Fig. 2), occur simultaneously on both curves.

The months of October, November and December, 1929, may be taken as another example showing that not only the maxima and minima of both curves may correspond to each other, but also that characteristic inter-

ruptions in the trend may be observed, such as could happen only in the case of cause and effect. On figure 3 the upward trend T_1 repeats itself in T_2 and T_3, after the ruptures R_1 and R_2 and the well-pronounced maxima $M \odot C^t$ and Mf display perfectly the cause and its effect, in this case belated by two days.

It seems evident that if the curves are not more similar than they are, many not perfect or even unsatisfactory solar constant values may be the cause of it.

These comparisons justified taking into consideration mean values. Therefore, for the years 1926 to 1930, I took all the maxima of solar con-



Time relations between maxima and minima in the solar constant and solar faculae

FIGURES 4 AND 5

Means of faculae and solar constants for the 5 days before and the 5 days after the dates of 72 selected days of maxima and 82 days of minima of solar constants.

stant values, preceded by increasing and followed by decreasing values for at least three days before and after. The same was done for the minima. The means of the 72 series of figures for the maxima and 82 for the minima give the diagrams on figures 4 and 5. Now, taking the values of the faculae for the same dates, we find (Fig. 5) that the mean minimum of faculae occurs one day after the mean minimum of solar radiation. The time difference between Greenwich and Washington makes it more than a day.

In the case of the mean maxima there is a difference of two days (Fig. 4). But considering the lines R_1 and R_2, between the third and the second days before the maximum of the solar constant, as discontinuities of a regular variation $m_1 M m_2$, we would have a coincidence of maxima. Therefore this

diagram induces the hypothesis that the solar atmosphere reacts against a full development of the faculae maximum, as it should have developed under the influence of the maximum of radiation. Nor is the minimum of faculae as deep as it should be.

In other words, the solar constant variations are not due to faculae, but the variations of the extent of faculae are due to the same variations of photospheric radiation as those of the solar constant.

A certain similarity to what occurs in our terrestrial atmosphere seems obvious: rain and hail subtract water from a thunderstorm cloud which is formed under the influence of strong ascending air currents; and, as we know, in the case of a minimum of such air chimneys, clouds may still be formed.

Do the solar constant variations affect the meteorological phenomena observed in our atmosphere?

For years Dr. Abbot has advocated such a thesis.

Meteorologists, in general, had doubts. I also was not entirely convinced, before the last few months of research work. But now I have the necessary data to prove, at least to my own satisfaction, not only that the processes in the solar photosphere—which produce changes in the extent of faculae, observed from day to day or from one group of days to another—directly affect the measured values of the solar constant, but also that in our atmosphere the changes of solar radiation, expressed by the figures of solar constant variations, are the direct cause of anomalies in the distribution of temperature and of all the complexity of the meteorological phenomena depending on temperature anomalies.

[1] *Proc. Am. Phil. Soc.*, **4**, 175, Philadelphia (1847).
[2] *M. N. R. Astr. Soc.*, **37**, 5, London (1877).
[3] *M. N. R. Astr. Soc.*, **55**, 460, London (1895).
[4] *A. N.*, **130**, 146, Kiel (1892).
[5] *A. N.*, **140**, 300, Kiel (1896).
[6] *Ann. Astroph. Obs. Smiths. Inst.*, **3**, 159, Washington (1913).
[7] *Smiths. Misc. Coll.*, **66**, No. 5, 18–19, Washington (1917).
[8] *C. R.*, **161**, 434, 485, Paris (1915).
[9] "Results of measures made . . . of Photographs of the Sun at Greenwich, the Cape and Kodaikanal in the year . . . "
[10] *C. R.*, **163**, 665–667, Paris (1916).
[11] *Ann. Rept. Smiths. Inst. for 1913*, p. 182.
[12] Loc. cit., p. 666.
[13] C. G. Abbot, *Smiths. Misc. Coll.*, **77**, No. 5, 23, Washington (1925).
[14] *Smiths. Misc. Coll.*, **77**, No. 6, 53, Washington (1925).
[15] *Ann. Astroph. Obs. Smiths. Inst.*, **5**, 279–285, Washington (1932).

THE COURSE OF THIAMIN METABOLISM IN MAN AS INDICATED BY THE USE OF RADIOACTIVE SULFUR*,†

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Communicated April 23, 1940

When supplementary thiamin (Vitamin B_1) is ingested or injected, the amount which appears in the urine during the succeeding twenty-four hours usually increases, but the total additional excretion always falls short of the supplement, even when the vitamin is injected and there can be no question of incomplete absorption. We have sought information on this unaccounted-for moiety by synthesizing thiamin from sulfur which contains the radioactive isotope S^{35} (designated as B_1*) and by following, after injection of the B_1*, the excretion of the radiosulfur (S*) in the urine and feces and the excretion of total free B_1 in the urine.

Radiosulfur was prepared by bombarding elementary sulfur with 7.5 MEV. deuterons, and thiamin bromide hydrobromide containing S^{35} was synthesized from the bombarded sulfur.[1]

The half-life of radiosulfur is about 88 days and it emits negative electrons with a maximum energy of 0.107 MEV.[2] This long half-life makes extended observations possible; some samples showed measurable activity 18 months after the radiosulfur had been prepared.

The radioactive vitamin was injected intramuscularly in two series of experiments; in the first series the subject, a young man in good health, was on a vitamin B_1-free diet for 36 days prior to the first injections and for the 15 days of the experimental period; in the second series the same subject was on a normal diet. Essentially the same results were obtained in both series.

Free (unphosphorylated) vitamin B_1 was determined in the urine by a modification of the thiochrome method. The urinary sulfur compounds were fractionated into the inorganic sulfate, ethereal sulfate and neutral sulfur components, the sulfur of each component was converted into elementary sulfur,[3] and the radioactivity of the sulfur was then measured quantitatively by comparison with a standard sample of the same radiosulfur. In the feces only the total radiosulfur was determined.

The radioactivity measurements were made with open, coincidence Geiger counters. The sulfide-coated copper counter tubes and the samples were enclosed in a large partially evacuated bell jar, provided with an externally operated arrangement for moving the samples successively to a definite position in front of the counters. The standard Rossi coincidence

circuit was used in the amplifiers; these had time constants of 3×10^{-4} seconds. It was possible to measure samples as weak as 3% of the background by making very long runs.

Separate experiments showed that B_1* added to urine does not exchange its radiosulfur with the inorganic or ethereal sulfur compounds; the S* was quantitatively recovered from the neutral sulfur fraction.

Figure 1 shows the daily excretion of radiosulfur (all forms) in the urine and feces after a daily intramuscular injection of 16 mg. of B_1* for four

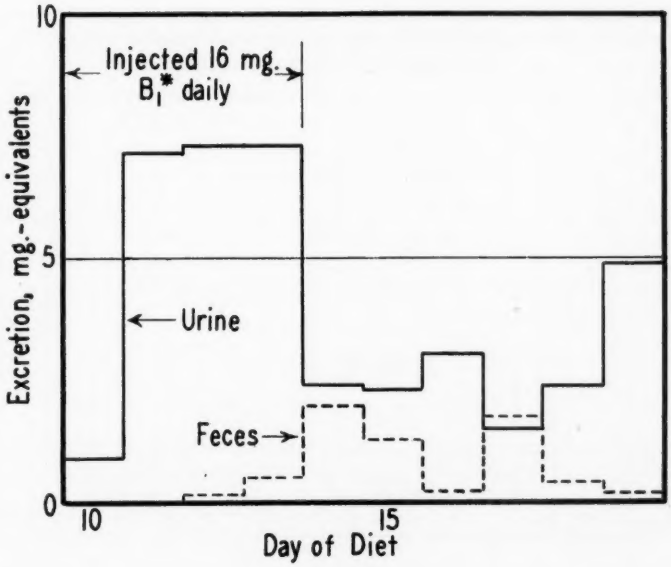

FIGURE 1

The daily recovery of radiosulfur (calculated as mg. of vitamin B_1) after intramuscular injection of B_1*; normal diet.

days, while the subject was on the normal diet. Six days after the last injection a total of 61% of the injected radiosulfur had been recovered from the urine and 11% from the feces; 28% of the injected material remained unaccounted for. It is clear from the low and diminishing excretion in the feces that the urine is the major excretory medium of parenterally administered B_1 and its decomposition products.

Figure 2 shows the daily excretion of free thiamin in mg. and of the radiosulfur present in the neutral sulfur fraction expressed in terms of mg. of B_1*. If only pure B_1* is present in urine these two should be the same;

consequently the excess of thiamin over radiosulfur shown in the figure indicates excretion of thiamin already present in the body. Since the total amount of B_1 in the blood in this case could hardly have exceeded 1 mg.[4] the excess of 7.5 mg. of non-radioactive vitamin in the urine on the first day of the injections must have come from the tissues. This indicates that the injected vitamin interacts very rapidly with that preëxisting in the tissues.

Although the whole of the neutral radiosulfur in the urine during the injection period shown in the figures may have been vitamin B_1, this is im-

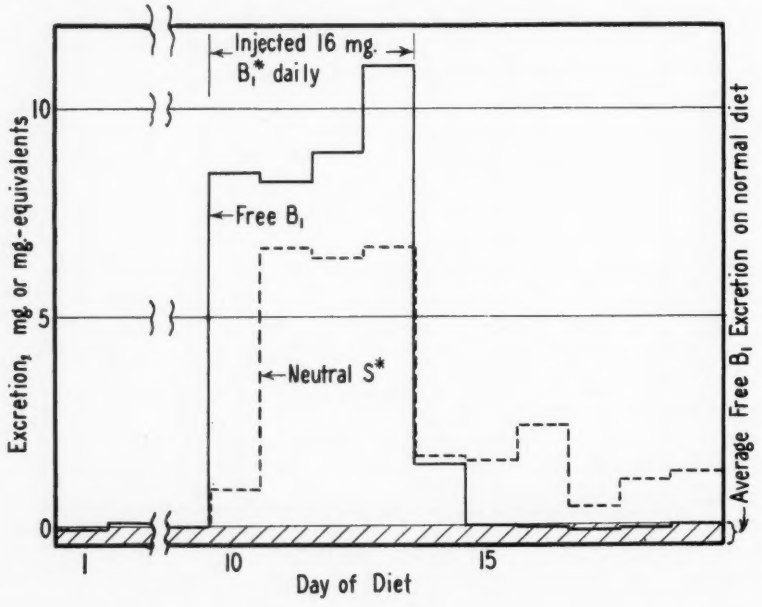

FIGURE 2

The daily excretion of neutral radiosulfur and free thiamin in the urine after intramuscular injection of B_1*; normal diet. The radiosulfur has been calculated in terms of mg. of vitamin B_1.

probable in view of the findings on the later days. The difference between the total free B_1 and the total neutral radiosulfur is certainly the minimum amount of preëxisting vitamin which was displaced from the tissues.

During the six days following the termination of the radiothiamin injections the neutral radiosulfur in the urine exceeded the thiamin excretion. The difference between the neutral radiosulfur and the free B_1 sulfur is the minimum amount of neutral sulfur-containing decomposition products of the thiamin which was excreted. The continued excretion of radio-

sulfur is evidence that tissue thiamin is continuously undergoing destruction at a rapid rate.

These features were shown even more clearly in the series of experiments when the subject was on the B_1-free diet and 2.7 mg. of B_1* were injected daily from the 37th to 46th days, inclusive, of the B_1-free diet (Fig. 3). Thiamin excretion in the urine increased immediately, but no radiosulfur was detected in the urine for the first two days (less than 20 γ). The entire increment in the urine came from preëxisting thiamin in the tissues which was displaced by the injected material, again indicating that injected B_1

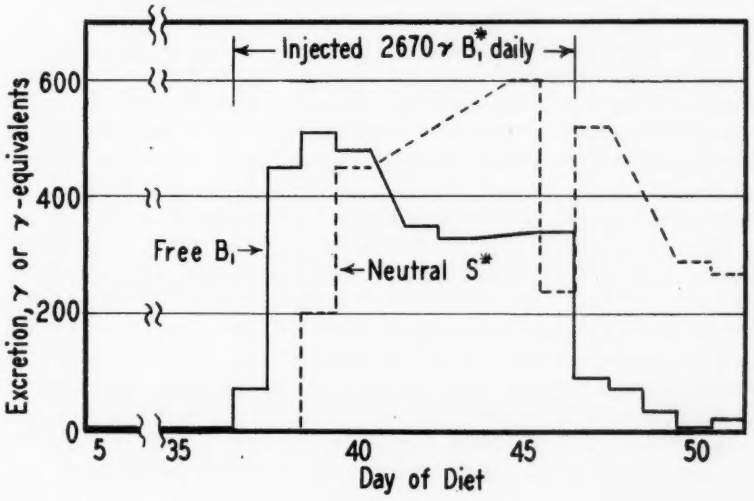

FIGURE 3

The daily excretion of neutral radiosulfur and free thiamin in the urine after intramuscular injection of B_1*; B_1-free diet. The radiosulfur has been calculated in terms of mg. of vitamin B_1.

rapidly enters the tissues from the blood. Since no thiamin was detectable in the urine for 30 days prior to the injections, the total quantity in the blood must have been less than 0.2 mg. For a short time at least after the injection of 2.7 mg., the ratio of radiothiamin to normal thiamin in the blood was probably greater than 1. If the excreted vitamin is an "overflow" moiety excreted before it enters the tissues, it should have been accompanied by a corresponding radiosulfur activity, but this was not found to be the case. The injected thiamin, therefore, must have entered the tissues very rapidly and interacted with the considerable quantities of thiamin already present.

Table 1 is a summary of some of the data illustrating this feature of thiamin metabolism. It is interesting that any free thiamin was displaced from the tissues of a subject who had been for 36 days on a B_1-free diet.

TABLE 1

THE DISPLACEMENT OF PREËXISTING THIAMIN FROM THE TISSUES BY ADDITIONAL THIAMIN INJECTED INTRAMUSCULARLY

DIET	INJECTION AND EXCRETION PERIOD (DAYS)	THIAMIN INJECTED (MG.)	MINIMUM AMOUNT OF PREËXISTING THIAMIN EXCRETED (MG.)
B_1-Free	3	8	0.8
Normal	1	16	7.5
Normal	4	64	16.0

The neutral sulfur and thiamin data in the two experiments are summarized in figure 4 which shows the difference between the thiamin and the neutral radiosulfur excretion. During the injection period the deficit of neutral radiosulfur indicates the minimum amount of preëxisting vitamin displaced from the tissues; the excess of neutral radiosulfur found in the urine after stopping the injections represents destroyed vitamin.

Figure 5 summarizes the urinary excretion of radiosulfur as inorganic sulfate; the radioactive sulfur appeared in this form in small quantities on the second day of the injections, and increased to considerable amounts by the end of the experiment, which is proof of the destruction of the injected vitamin and oxidation of the thiazole ring. The SO_4* from this oxidation mixes with the large amount of SO_4 in the body;[5] the excretion of the radiosulfur is therefore delayed. The oxidation of the thiazole ring was therefore probably much more extensive than is indicated by the amount of SO_4* in the urine in the first few days after beginning the injections.

The amounts of radiosulfur found as ethereal sulfate were in most cases very close to the experimental error of the radioactivity measurements; only a very small fraction of the radiosulfur appears in this form, and it may have arisen from exchange with the inorganic sulfate.

The extensive destruction of B_1 in the body is indicated in table 2.

TABLE 2

DESTRUCTION OF INTRAMUSCULARLY INJECTED THIAMIN

	TOTAL RECOVERY OF S* FROM B_1* (% OF TOTAL INJECTED)		PER CENT OF RECOVERED S* IN THE URINE WHICH REPRESENTS DESTROYED VITAMIN		
	FECES	URINE	AS NEUTRAL S COMPOUNDS	AS INORGANIC SULFATE	TOTAL
B_1-Free	..	26	21	21	42
Normal	11	61	18	25	43

Summary.—1. There is a rapid interaction of injected B_1 with that present in the blood and tissues.

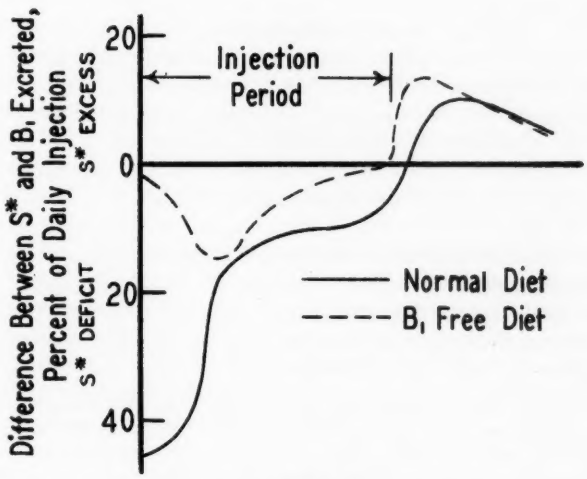

FIGURE 4

The difference between neutral radiosulfur and thiamin excretion after the injection of B_1*.

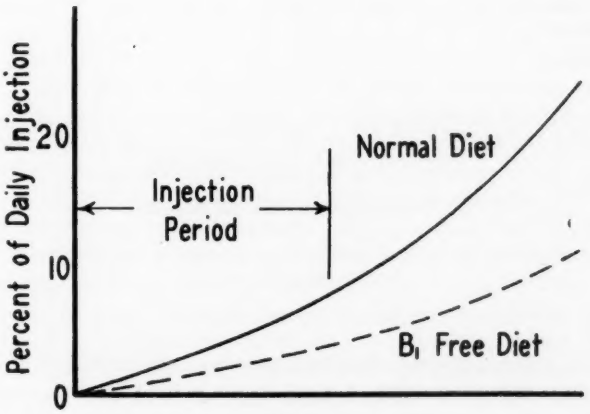

FIGURE 5

The urinary excretion of radiosulfur as inorganic sulfate after the injection of B_1*.

2. The displacement of preëxisting thiamin by injected thiamin demonstrates that a significant amount of the vitamin remains in the tissues even after 36 days of a B_1-free diet; larger quantities are present under normal nutritional conditions. This does not imply that the amount retained after a prolonged B_1-free diet is an adequate protective amount of this vitamin.

3. The metabolism (interchange and destruction) of vitamin B_1 is rapid and thus resembles that of the main metabolites—protein, fat and carbohydrate.

4. The rapid destruction of thiamin yields in the urine neutral sulfur compounds and inorganic sulfate.

5. The losses incurred by excretion and destruction are inevitable in the maintenance of a physiologically adequate concentration of thiamin and cocarboxylase in the blood and tissues.

* This work was aided by grants from the National Research Council, the Research Corporation, the Hixon Fund and the Globe Mills Fund (California Institute of Technology).

† This paper was presented at the meeting of the American Society of Biological Chemists in New Orleans, March 14–16, 1940.

[1] Cf. E. R. Buchman, *Jour. Am. Chem. Soc.*, **58**, 1803 (1936); J. K. Cline, R. R. Williams and J. Finkelstein, *Ibid.*, **59**, 1052 (1937).

[2] W. F. Libby and D. D. Lee, *Phys. Rev.*, **55**, 245 (1939).

[3] R. A. Cooley, Don M. Yost and Edwin McMillan, *Jour. Am. Chem. Soc.*, **61**, 2970 (1939).

[4] Robert Goodhart and H. M. Sinclair, *Jour. Biol. Chem.*, **132**, 11 (1940).

[5] Henry Borsook, Geoffrey Keighley, Don M. Yost and Edwin McMillan, *Science*, **86**, 525 (1937).

RADIOACTIVE CARBON IN THE STUDY OF RESPIRATION IN HETEROTROPHIC SYSTEMS

By S. Ruben and M. D. Kamen

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It is now well established[1] that the presence of small amounts of CO_2 are indispensable for the growth of many types of heterotrophic organisms. Also it has been reported[2] that the reduction of methylene blue by certain bacteria is dependent upon traces of CO_2. It appears that some of the experiments we have been doing with radioactive carbon have direct bearing on these interesting results.

Several heterotrophic (non-photosynthetic) systems, namely, yeast (bakers'), *B. coli*,[3] ground plant (barley) roots, ground liver (rat) tissue, etc., have now been found to assimilate small quantities of radioactive carbon as CO_2. This assimilation is inhibited by the presence of HCN. Since in the respiratory processes of these heterotrophic cells there is a net production of CO_2 it is clear that a CO_2 assimilation can best be studied directly by isotopic tracer methods using either stable (C^{13}) or radioactive (C^{11} and C^{14}) carbon.

It is also apparent that the presence of radiocarbon in oxidation states lower than +4 does not necessarily mean a net reduction of CO_2 has occurred. It may be due to the existence of a reversible reaction involving CO_2 as an end-product. At the present time no such reversible reactions in the respiratory process are known. It seems reasonable in view of the work of Hes and others that the formation of reduced radiocarbon by a respiring cell is due not to a simple interchange but rather that CO_2 plays the rôle of a highly specific oxidizing agent.

It would seem that the entrance of a CO_2 molecule into the system is necessary for the production of an additional large number of CO_2 molecules. In a sense, then, respiration is auto-catalytic.

It has been most convenient to work with yeast and the results described below have been obtained with fresh yeast cells suspended in distilled water. The suspensions were exposed to a C^*O_2 (0.5 to 2 cm.)-air mixture at approximately atmospheric pressure. Since short lived C^{11} (21-minute half-life) was used the exposures were of short duration (5 to 100 minutes). At various intervals $NaHCO_3$ was added to the suspensions, which were then boiled vigorously with strong acid to remove dissolved C^*O_2. The method of measuring the activity has been described elsewhere.[4] The uptake of C^*O_2 by aqueous suspensions of yeast cells as a function of length of time of exposure to C^*O_2 is shown in figure 1.

The measurements are, of course, corrected for radioactive decay, and are therefore comparable. 10^7 counts/min. corresponds to 0.01 cc. C^*O_2 (S. T. P.). The rate of CO_2 production by each suspension was 0.01 cc. (S. T. P.) CO_2 per minute. Thus under these conditions one C^*O_2 molecule was reduced for every 50 molecules produced in respiration. These figures are to be considered merely semi-quantitative. A constant rate of C^*O_2 reduction is obtained only when the amount of C^*O_2 present at the start of the experiment is large compared to the CO_2 evolved during the exposure. If this is not the case then the C^*O_2 is diluted, and in addition lack of rapid equilibration between the freshly produced CO_2 within the cell and the C^*O_2 in the rest of the vessel becomes important. Thus at high respiratory rates the accumulation of reduced radioactive carbon may be less.

Attempts to chemically identify the active molecules have thus far been unsuccessful. When the cells are boiled with dilute acid for approximately

one minute the cell-free aqueous extract contains more than 90% of the reduced C*. If the boiling acid treatment is omitted the cell-free medium has only ~1% of the activity. Osazones prepared with phenyl hydrazine as well as hydrazones of 2,4 dinitro phenylhydrazine are inactive (<1% of the activity). In the chemical analyses a mixture of aldehydes, sugars, acids, etc., was added to furnish carriers. Special attention was centered on pyruvic acid since the decarboxylation of pyruvic acid ($CH_3C(=O)C(=O)OH = CO_2 + CH_3CHO$) is known to play an important part in catabolic reactions. That the pyruvic acid fraction was inactive (<0.5%) indicates this reaction is irreversible. Salts of Ba^{++} precipitated in 80% ethanol were very active and even after several reprecipitations contained ~50% of the radioactivity. Decarboxylation of the active Ba salts was attempted at 250°C. for one hour. Only ~3% of the C* was converted to $BaCO_3$ by this treatment.

It is of interest at this point to mention that a non-photochemical[5] reduction of CO_2 is carried out byg reen plants (barley, wheat, sunflower, chlorella, etc.). This seems to be definitely a part of the photosynthetic mechanism.[6] The dark pick-up of CO_2 by plants has also been observed and measured by other investigators using different methods.[7, 8]

The evidence accumulated thus far indicates differences exist between the dark C^*O_2 reduction by a photosynthetic (chlorella) system on one hand and a non-photosynthetic (yeast) on the other. The time course of the C^*O_2 dark reduction by chlorella is shown in figure 2 and is to be contrasted with figure 1.

Decarboxylation experiments (dry distillation of the Ba salt at 250° for 1 hour) on the active material from chlorella suggest the major part of the C* is present in a —COOH group. Furthermore the radioactive molecules formed in yeast were found in diffusion experiments to have a higher diffusion coefficient (and are very likely of lower molecular weight) than the active compounds formed in chlorella. The dark reduction of C^*O_2 by chlorella is reversible. It is not certain whether the C^*O_2 uptake by yeast is reversible. If the yeast cells are allowed to react with C^*O_2 for 60 minutes and then flushed with a continuous stream of N_2 for 30 minutes, there is no decrease in the reduced C* present in the cells. If a stream of (inactive) CO_2-N_2 (50–50 mixture) is used, then ~15% of the reduced carbon is removed. Whether this loss occurs via the same path as the C^*O_2 reduction or by means of other reactions is still uncertain. In any case it is now an accepted fact that CO_2 reduction is no longer an exclusive characteristic of photosynthetic and chemosynthetic autotrophic organisms.[9]

It is conceivable, as Professor G. N. Lewis and Professor W. C. Bray

have suggested to us, that the primary reactions resulting in CO_2 reduction may be similar in many respects for photosynthetic and non-photosynthetic systems. The secondary reactions may differ enormously in the autotrophic as compared to the heterotrophic organisms, since in the former the net reaction is an accumulation whereas in the latter it is combustion of organic matter. It may prove easier to investigate the photosynthetic primary step because the secondary reactions take place only in the light. This is not the case in the heterotrophic systems since one must deal with a steady state rather than an equilibrium condition.

In conclusion, then, experiments with radioactive carbon have shown that a number of heterotrophic systems reduce small amounts of C^*O_2. The chemical identity of the active molecules is thus far unknown. These results offer positive evidence that CO_2 is a specific oxidizing agent in

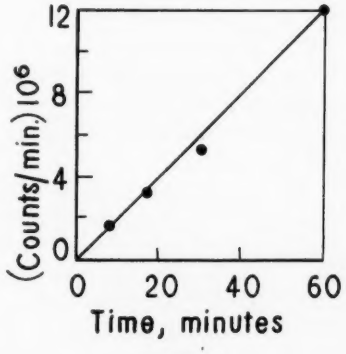

FIGURE 1

C^*O_2 assimilation by yeast.

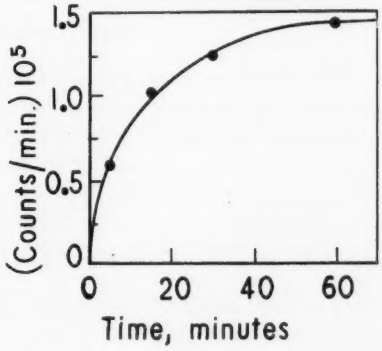

FIGURE 2

Dark C^*O_2 reduction by chlorella.

respiratory processes. This suggestion was first proposed by several investigators as a possible explanation for the fact that small quantities of CO_2 are essential for growth for microörganisms.[1]

It seems certain that further experiments with radiocarbon will yield important information regarding the mechanism.

We wish to thank Professor E. O. Lawrence for his interest and Dr. W. Z. Hassid for assistance in some of the experiments. We are indebted to Professor G. N. Lewis, Professor W. C. Bray and Professor H. A. Barker for helpful discussions and suggestions. Thanks are also due the Rockefeller Foundation for financial support to the Radiation Laboratory.

[1] For a summary, see Hes, *Ann. Fermentation*, **4**, 547 (1938).

[2] Hes, *Nature*, **141**, 647 (1938).

[3] We are indebted to Professor C. B. van Niel for the coli and Mr. A. R. Robinson for the preparation of the liver tissue.

[4] Ruben, Hassid and Kamen, *Jour. Am. Chem. Soc.*, **61**, 661 (1939).
[5] Ruben, Kamen, Hassid and DeVault, *Science*, **90**, 570 (1939).
[6] Additional evidence to be published shortly.
[7] McAlister, *Jour. Gen. Physiol.*, **22**, 613 (1939).
[8] Emerson and Lewis, *Am. Jour. Botany*, **26**, 808 (1939).
[9] Cf. van Niel, *Ann. Rev. Biochem.*, **6**, 606 (1937); Gaffron, *Ibid.*, **7**, 986 (1939).

CO_2 ASSIMILATION BY PROPIONIC ACID BACTERIA STUDIED BY THE USE OF RADIOACTIVE CARBON

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The studies of Wood and Werkman[1, 2, 3] as well as the work of Phelps, Johnson and Peterson[4] have shown that propionic acid bacteria can utilize CO_2 during the fermentation of glycerol. In the absence of CO_2 this fermentation can be adequately represented by the equation:[5]

$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} = \begin{array}{l} CH_3 \\ | \\ CH_2 \\ | \\ COOH \end{array} + H_2O$$

In the presence of CO_2 the formation of propionic acid is accompanied by the appearance of succinic acid in amounts closely equimolar with the quantity of absorbed CO_2.

This made it seem possible that CO_2 becomes converted into succinic acid by combination with a 3-carbon compound. The formation of succinic acid during the fermentation of pyruvate, dextrose and galactose by *Escher. coli*, particularly its dependence upon the CO_2 partial pressure,[6] supports this view.

It is apparent that important information regarding the mechanism through which CO_2 is utilized can be obtained by the use of radioactive CO_2.[7] We have employed this approach in a study of the fermentation of glycerol by *Propionibacterium pentosaceum*.

The bacteria were grown anaerobically in yeast extract-glycerol media in the presence of CO_2 for 3 to 6 days. For the experiments cells from 250 to 500 ml. of such cultures were centrifuged, washed and suspended in 0.5 per cent phosphate buffer at pH 7.0 with and without added substrates. The suspensions were shaken at 30°C. in the presence of N_2 and C^*O_2[8] for

30 to 40 minutes. After centrifuging off the cells, propionic acid and succinic acid were added to the supernatant liquid to provide carriers for the small quantities of radioactive metabolic products. The volatile and non-volatile acids were separated by exhaustive steam distillation; the cells were boiled gently with acid for a few minutes and separated from the acid extract by centrifugation. Special care was taken to remove all traces of dissolved C^*O_2 before radioactivity measurements were made. The results of an experiment carried out in the presence of 2 per cent glycerol are summarized in table 1.

TABLE 1

BACTERIA + 2 PER CENT GLYCEROL + C^*O_2 FOR 40 MINUTES

	PER CENT OF TOTAL C* ASSIMILATED[a]
Volatile acids	72.0
Non-volatile acids	10.0
Boiled cell extract	17.5
Cells after extraction	0.5

[a] All measurements are corrected for decay and are therefore comparable.

The above table shows that 72 per cent of the C^*O_2 taken up by the cells can be recovered in the volatile acid fraction and only 10 per cent in the non-volatile acid fraction. The boiled cell extract consisted mainly of acids carried down with the cells.

The activity in the volatile fraction was found to be restricted to propionic acid by a Duclaux distillation. The C* content of successive distillates is compared in table 2 with the titration values obtained with pure propionic acid distilled under the same conditions.

TABLE 2

PER CENT VOLUME OF DISTILLATE	PER CENT RADIOACTIVITY FOUND	PER CENT ACIDITY BY TITRATION FOR PURE PROPIONIC ACID
20.0	23.0	24.5
40.0	45.0	46.7
60.0	65.0	67.0
80.0	84.0	85.1
100.0	100.0	100.0

The agreement between the radioactivity and titration measurements is remarkably good. This result excludes the presence of more than 5% of C* in other fatty acids (formic, acetic, etc.).

The radioactive component of the non-volatile acid fraction was identified as succinic acid in two ways. The distribution coefficients of the non-

volatile material between water and ethyl ether by titration and radioactivity measurements are compared in table 3.

TABLE 3

DISTRIBUTION COEFFICIENTS
(10 Ml. Aqueous Phase + 90 Ml. Ether)

	TITRATION	RADIOACTIVITY
K	7.4	6.0

In addition, the non-volatile material was sublimed and the specific activity $\left(\frac{C^*}{\text{succinic acid}}\right)$ of the various fractions is shown in table 4.

TABLE 4

SUBLIMATION

FRACTION	SPECIFIC ACTIVITY $\left(\frac{\text{RADIOACTIVITY}}{\text{SUCCINIC ACID}}\right)$
1 (210–220°C.)	9.5
2 (220–240°C.)	9.9

No radioactive material came off below 210°C. It seems quite reasonable to conclude that the radioactivity is mainly due to succinic acid.

The remarkable result that so much of the radioactive carbon is found in propionic acid might be explained by assuming a reversible reaction $CH_3CH_2COOH + CO_2 \rightleftarrows COOHCH_2CH_2COOH$. Experiments were performed to test this hypothesis by the addition of propionic acid and succinic acids to cell suspensions in the presence of C^*O_2. In order to reduce the amount of glycerol and reserve products in the cell material, the bacteria were centrifuged from the medium in which they were grown, and suspended in phosphate buffer for 3 hours; this procedure was then repeated, and immediately before exposure to C^*O_2 they were again washed. The results of this experiment are shown in table 5.

TABLE 5

CELL SUSPENSION IN PHOSPHATE BUFFER EXPOSED TO C^*O_2 FOR 30 MINUTES

SUBSTRATE	TOTAL C^* ASSIMILATED (ARBITRARY UNITS)	RADIOACTIVITY OF $\frac{\text{VOLATILE ACID}}{\text{NON-VOL. ACID}}$
Nothing	5.3	0.7
0.4 per cent Na propionate	4.8	0.78
0.2 per cent Na succinate	4.5	0.2
2.0 per cent glycerol	100.0	3.1

In a further experiment the cells were suspended in phosphate buffer for 7 hours and then centrifuged and washed as above. The results are summarized in table 6.

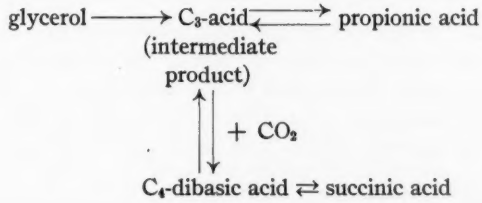
TABLE 6

CELL SUSPENSION IN PHOSPHATE BUFFER EXPOSED TO C^*O_2 FOR 30 MINUTES

SUBSTRATE	TOTAL C^* ASSIMILATED (ARBITRARY UNITS)	RADIOACTIVITY OF VOLATILE ACID / NON-VOL. ACID
Nothing	2.3	0.41
2 per cent glycerol	100.0	3.0, 3.8
2 per cent glycerol + 0.4 per cent Na propionate	85.0	2.4

The addition of propionic acid in the absence of glycerol has little if any effect on the distribution of the C^* between the propionic and succinic acids or on the total C^* taken up. This is evidence against the formation of succinic acid from CO_2 and propionic acid. The presence of glycerol not only has an enormous effect on the C^*O_2 uptake, but also increases the ratio of radioactive carbon in propionic acid and succinic acid. It seems quite likely in view of these results that propionic and succinic acids are formed via reactions between CO_2 and glycerol or intermediate products arising during its fermentation. Pyruvic acid, which could be a possible intermediate, was found to be inactive ($<0.5\%$).

The data so far presented could find an explanation on the basis of a mechanism such as:



In this case one would expect the radioactive carbon atom to be located in the carboxyl groups. Further experiments are in progress to test this hypothesis.

The results of the above experiments are of general interest also in connection with a major problem encountered in tracer experiments with labeled carbon, namely, the synthesis of radioactive molecules starting with C^*O_2. In many cases synthetic organic methods result in poor yields despite time-consuming and painstaking effort. In such instances the use of appropriate microörganisms to perform the desired synthesis may be highly desirable. We may cite the above experiments as an example. In 30 minutes the bacteria converted over 80 per cent of the assimilated C^*O_2 into propionic and succinic acids, which are thus made readily available for tracer experiments.

It is a pleasure to thank Dr. M. D. Kamen and Professor E. O. Lawrence

of the Radiation Laboratory for their interest, and for making the radioactive carbon available. We are indebted to Professor C. B. van Niel and Professor H. A. Barker for much advice and assistance.

[1] *Biochem. Jour.*, **30**, 48 (1936).
[2] *Ibid.*, **32**, 1262 (1938).
[3] *Ibid.*, **34**, 7 (1940).
[4] *Biochem. J.*, **33**, 726 (1939).
[5] C. B. van Niel, *The Propionic Acid Bacteria*, Haarlem (1928).
[6] Elsden, *Biochem. Jour.*, **32**, 187 (1938).
[7] Ruben, Hassid and Kamen, *Jour. Am. Chem. Soc.*, **61**, 661 (1939).
[8] The symbol C* will be used to represent carbon labeled by the radioactivity of some of its atoms. The isotope is C^{11} and was produced in the Berkeley cyclotron; it has a half-life of 21 minutes.

THE REDUCTION OF RADIOACTIVE CARBON DIOXIDE BY METHANE-PRODUCING BACTERIA

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Several years ago[1] it was shown that the formation of methane in the fermentation of ethyl and butyl alcohols and of butyric acid by impure cultures of methane-producing bacteria is accompanied by the disappearance of an equi-molecular quantity of carbon dioxide. In the fermentation of ethyl alcohol, for example, the reaction can be rather closely described by the equation:

$$2C_2H_5OH + CO_2 \longrightarrow 2CH_3COOH + CH_4 \qquad (1)$$

The same reaction has recently been shown[2] to be carried out by pure cultures of *Methanobacterium Omelianskii*.

As to the mechanism of the above reaction, it seemed quite likely that the acetic acid arises by an oxidation of ethyl alcohol, carbon dioxide being simultaneously reduced to methane. This would be in accordance with the view originally advanced by Professor C. B. van Niel that the methane fermentation of organic as well as of inorganic compounds is a process of oxidation by means of carbon dioxide which can be represented by the general equation:

$$4H_2A + CO_2 \longrightarrow 4A + CH_4 + 2H_2O \qquad (2)$$

where H_2A is an oxidizable molecule and A is its oxidation product. Conclusive proof in favor of this mechanism, however, was lacking. Furthermore, no direct experimental evidence could be obtained in support of the

reduction of carbon dioxide in those very numerous methane fermentations of organic compounds which result in the formation of carbon dioxide. An example of such a process is the fermentation of methyl alcohol, the net reaction for which is

$$4CH_3OH \longrightarrow CO_2 + 3CH_4 + 2H_2O \qquad (3)$$

This reaction could—in accordance with the van Niel hypothesis—be the result of a complete oxidation of four molecules of methyl alcohol to carbon dioxide accompanied by a reduction of three molecules of carbon dioxide to methane. But such a reduction of carbon dioxide could obviously not be observed by gross chemical analysis.

Until recently there seemed no immediate hope that the applicability of the van Niel hypothesis to methane fermentations producing carbon dioxide could be tested. The production of intense samples of radioactive carbon in the Radiation Laboratory of the University of California[3, 4] has, however, made possible a direct and relatively simple test for carbon dioxide reduction even in such fermentations. For if radioactive carbon in the form of carbon dioxide is supplied to organisms fermenting organic compounds the methane produced must also be radioactive if the van Niel hypothesis is correct. If it is incorrect the methane will be inactive.

Radioactive carbon* experiments have been carried out with pure cultures of two species of methane-producing bacteria, *Methanobacterium Omelianskii* and *Methanosarcina methanica*.† The former organism is able to oxidize simple primary and secondary alcohols only as far as the corresponding fatty acids and ketones, respectively, and is known from chemical evidence to reduce carbon dioxide in accordance with equation (2). The latter organism oxidizes methanol, acetic acid and probably other compounds completely to carbon dioxide, so preventing direct chemical observation of carbon dioxide utilization.

The experiments were carried out with heavy cell suspensions prepared by centrifuging bacteria from 1–2 liters of medium, washing and resuspending in 10–15 cc. of a phosphate buffer solution (pH 7.0) containing 0.01% $Na_2S.9H_2O$. The final suspensions contained 1.0–1.2 mg. of cell nitrogen or about 10–12 mg. dry weight of ash-free cells per cubic centimeter.

Experiments with Mb. Omelianskii.—The experiments with *Mb. Omelianskii* were undertaken with two objectives. The first was to obtain direct evidence concerning the reduction of carbon dioxide to methane. As has already been mentioned, the chemical evidence already very strongly indicated such a reaction. The second objective was to find out whether carbon dioxide is also converted into cell material. Carbon balance experiments described elsewhere[2] have shown that the synthesis of cell material in media containing ethanol and carbon dioxide occurs in one of two ways. Either (1) the cell material is derived exclusively from ethanol, carbon

dioxide being converted only into methane, or (2) the cell material is derived both from ethanol and carbon dioxide. A decision between these two possibilities can be reached by determining whether or not radioactive carbon dioxide gives rise to radioactive cell material.

For the experiments suspensions of bacteria grown in an ethanol-bicarbonate medium were used. The bicarbonate supply was adjusted so as to be limiting; at the end of the fermentation, the growth medium was free of carbon dioxide. Two experimental vessels were used, both of which contained 5 cc. of cell suspension plus 0.5 cc. of 8% (vol.) ethanol. To one vessel was added 0.5 cc. of a 10% $HgCl_2$ solution. Both suspensions were incubated for 40 minutes at 40°C. after adding the radioactive carbon dioxide. At the end of the incubation period the gases were pumped out of the vessels and the residual carbon dioxide removed by absorption in alkali. The methane was then combusted by passing over hot copper oxide; the resulting carbon dioxide was absorbed in alkali. The radioactivity of the alkali carbonate solution was measured by means of a Geiger counter.

The cell suspension from each vessel was acidified to pH 1 with H_2SO_4 and steam-distilled to separate the volatile acid. The residuum was concentrated to dryness on a hot plate to free the cells of carbon dioxide; the radioactivity was then measured. Control experiments showed that carbon dioxide is completely removed by the preliminary acid treatment. The radioactivities of the cells and methane are given in table 1. The volatile acid fraction was inactive. The data in table 1 are, of course, corrected for decay to a convenient time so that they are directly comparable.

TABLE 1

REDUCTION OF CARBON DIOXIDE BY *Mb. Omelianskii*

(Activities are expressed in counts per minute. Background = 30 counts per minute)

EXPERIMENT	TOTAL ACTIVITIES OF METHANE	TOTAL ACTIVITIES OF CELLS
Living cells	12×10^4	18×10^2
Dead cells ($HgCl_2$)	...	0

The formation of radioactive methane in this experiment shows conclusively that carbon dioxide is reduced to methane by *Mb. Omelianskii*. The quantity of radioactive methane formed was sufficient to give a count of the order of 4000 times greater than the background count. The absence of any radioactivity in the volatile acid fraction provides direct evidence against the view that methane is formed via acetic acid.

The results also show that carbon dioxide is converted in easily demonstrable amounts into cell material by living cells. Dead cells are completely inactive. In connection with other evidence,[2] it must therefore be concluded that both ethanol and carbon dioxide are used in the synthesis of

cell material. The ratio of radioactivities of cells and methane indicates that about 1.5% of the carbon dioxide reduced is so used.

Experiments with Ms. methanica.—This organism was grown in a medium containing 0.6% (vol.) of methanol. When the fermentation had almost stopped, the bacteria were centrifuged and resuspended as described above. The action of cell suspensions was tested upon methanol and sodium acetate; the technique of the experiments was the same as in those of *Mb. Omelianskii*. Since the experiments were essentially qualitative in nature, only the general results will be described.

The experiments showed quite conclusively that *Ms. methanica* also is able to reduce carbon dioxide to methane. Radioactive methane was obtained from suspensions provided with methanol or with sodium acetate. However, since the quantity of radioactive gas was of the same order of magnitude in both cases and since the presence of small amounts of methanol (from the growth medium) in the acetate experiment could not be completely prevented, the evidence for the reduction of carbon dioxide to methane as a result of the oxidation of acetic acid remains inconclusive. The reduction of carbon dioxide to methane as a result of the fermentation of methanol can be considered definitely established.

Although in the fermentation of methanol, carbon dioxide is reduced to methane, it should be emphasized that the data obtained are not adequate to show whether or not *all* the methane originates in this way. It is also possible that the alcohol might be directly reduced to methane. Further experiments are required to elucidate this point

It has been found that *Ms. methanica* also utilizes carbon dioxide in the synthesis of cell material. The ratio of radioactivity of cells to methane is about 1:10. From this ratio and the equation for the fermentation of methanol, it can be estimated that the quantity of carbon dioxide assimilated is sufficient to account for at least a large part of the cell synthesis. The exact quantity of cell substance formed by this organism is not known, but anaerobic bacteria generally and *Mb. Omelianskii* in particular are known to assimilate only 5–10% of the total substrate decomposed.

Conclusions.—Although the above experiments deal with only two of the many kinds of methane-producing bacteria and with only three of the many organic compounds fermented by these organisms, they considerably strengthen the generalization that the methane fermentation of organic as well as of inorganic compounds is essentially an anaerobic oxidation process in which carbon dioxide acts as the ultimate hydrogen acceptor (oxidizing agent) and is reduced to methane. And it has been established that methane bacteria, like many other living organisms,[‡] have the ability to convert carbon dioxide into organic constituents of their cells.

This work was made possible by the invaluable coöperation of Professor E. O. Lawrence and members of the Radiation Laboratory. Financial aid

from the Rockefeller Foundation to the Radiation Laboratory is gratefully acknowledged.

Clerical assistance was furnished by the personnel of the Works Progress Administration official Project No. 65-1-08-91-B-10.

* C^{11} (21-minute half-life) was used in our experiments.

† The former organism was isolated by one of us (H. A. B.). The latter was isolated by Mr. Schnellen (Delft) and was obtained through the courtesy of Professor A. J. Kluyver. To both Mr. Schnellen and Profesor Kluyver the authors wish to express their appreciation.

‡ Unpublished experiments by Ruben, Hassid and Kamen have shown that many types of heterotrophic organisms and tissues including yeast, *B. coli*, propionic acid bacteria, liver (rat), algae and various parts of higher plants can reduce carbon dioxide in the dark. It seems quite likely that this is a general property of living organisms. See *Proc. Nat. Acad. Sci.*, **26**, 418–422 (1940).

[1] Barker, H. A., *Arch. Mikrobiol.*, **7**, 404–419 (1936).

[2] Barker, H. A., Unpublished.

[3] Ruben, S., Hassid, W. Z., and Kamen, M. D., *Jour. Am. Chem. Soc.*, **61**, 661–663 (1939).

[4] Ruben, S., Kamen, M. D., Hassid, W. Z., and DeVault, D. C., *Science*, **90**, 570–571 (1939).

THE CORONAVISER, AN INSTRUMENT FOR OBSERVING THE SOLAR CORONA IN FULL SUNLIGHT

By A. M. Skellett

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Read before the Academy, October 24, 1939

The old problem of attempting to observe the solar corona in full sunlight is not only of interest to astronomers but also to those radio engineers who are concerned with radio transmission over long distances. The major disturbances of such transmission have their origin in the sun and studies to date have indicated that a day to day knowledge of the activity of the corona might prove useful in predicting the transmission conditions.

The recent success of Lyot[1] leaves much to be desired in the way of a continuous record of coronal activity. A method of greater discrimination is needed. Such a method was proposed[2] several years ago and is based on the use of television technique, the corona being separated from the glare by electrical filters while the image of the sky around the sun is in the form of an electric current.

This is a report of the first practical trial of the method. Special television apparatus has been developed at the Bell Telephone Laboratories for use in conjunction with the 15″ horizontal refractor of the Cook Observatory[3] at Wynnewood, Pa. See figure 1. The input scanner is me-

chanical and scans a ring-shaped area in the sky around the sun, the flying spot tracing out the area along a spiral path. This scanner is shown in figure 2. The back-silvered lens L by rocking while it rotates effectively scans the sky image in the plane of the masking disc D with the scanning hole H. The apparatus at the other end of the motor shaft generates the

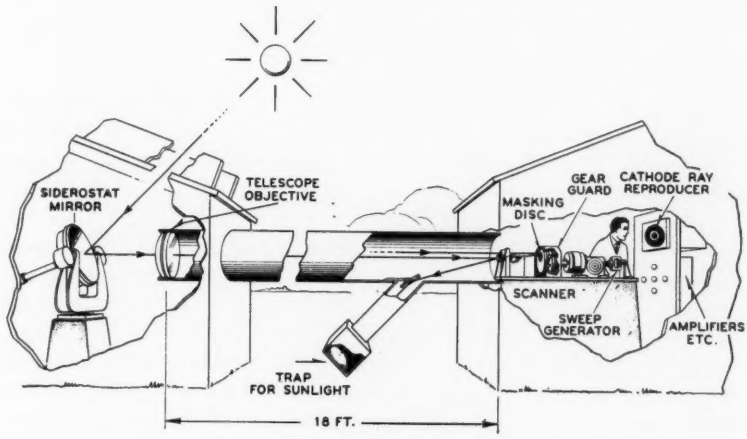

FIGURE 1
Layout of the apparatus at the Cook Observatory.

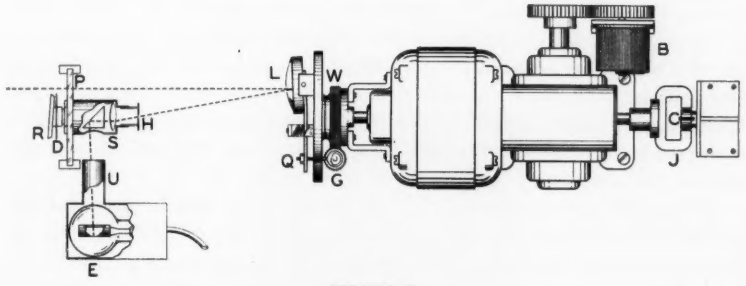

FIGURE 2
The input scanner and sweep voltage generator.

electrical waves which direct the electron beam of the reproducing cathode-ray tube around a path corresponding with that of the input. A detailed description of the technical details of all of the apparatus is published elsewhere.[4]

The glare of the clear sky is uniform angularly around the solar image and, therefore, as the scanning spot travels around the field, it gives rise

mainly to a direct current in the photocell. But the coronal features, that is, the streamers, arches, etc., generate a current of varying amplitude which amounts to an alternating component in the photoelectric current and only this latter component is amplified so that the reproduction contains, ideally at least, only the coronal image. Inaccuracies of alignment and nonuniformity of the intensity of the glare across the field occasioned by instrumental defects give rise to fairly strong low frequency components, particularly at the circular scan frequency or fundamental of the image signal. A high-pass filter whose cut-off characteristic could be altered was included in the circuit in order to eliminate such undesired signals. There was also included a low-pass filter to eliminate high frequency noise. The photocell (*E*, Fig. 2) had a caesium sulphide surface with a spectral characteristic that is a fairly close match to that of sunlight.

Since the inner corona has a surface brightness of about the same magnitude as the full moon, the sensitivity of the apparatus was checked by obtaining images of the moon in its various phases. These intensity levels are of the order of a millionth of that of the brightness of the solar surface. The magnitude of the glare through which it was necessary to work was of the order of 1000 times these values. This high level of glare was probably due to scattering by telescopic parts, particularly the siderostat mirror, rather than the sky. There were, however, many days when the glare from the sky had a magnitude considerably higher than this value.

Extreme care had to be taken to keep the optical parts of the apparatus clean. The slightest speck of dust on certain surfaces of the optical parts gave rise to overloaded images on the cathode ray tube, for the essence of the method is the amplification of minute variations in the intensity of illumination from point to point in the field. Occasionally tiny specks of brilliant light floated across the screen. These were finally traced to windborne seeds and insects which drifted across the sky between the apparatus and the sun.

It was necessary to have an absolute criterion by which one could distinguish between parasitic images which were caused by instrumental defects and coronal and prominence images which were associated with the sun. Such a test was furnished by the horizontal mounting of the telescope. By virtue of this arrangement, the celestial field rotates about the optic axis of the telescope with time. Photographs were taken at successive intervals over a period of several hours during which time the celestial images rotated through a sufficient angle (about 7° per hour) to distinguish them from the stationary parasitic images.

A large number of prominences have been observed and photographed with the apparatus. Figure 3 shows the prominences around the sun on October 31, 1938. This was taken with a red glass filter (Schott RG2)

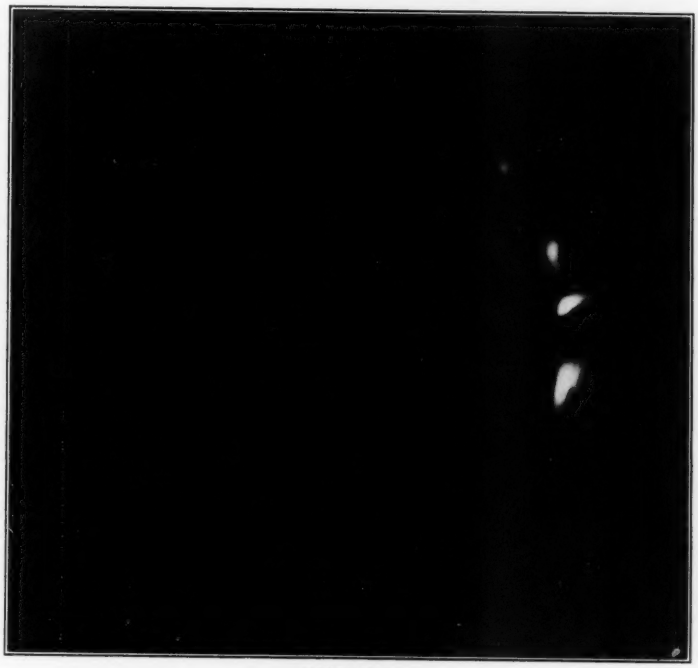

FIGURE 3

Prominences around the sun on Oct. 31, 1938.

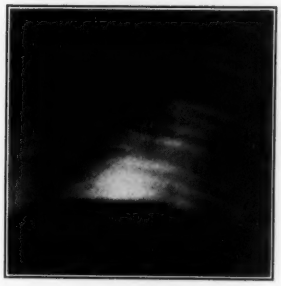

FIGURE 4

A jet or flare in the corona photographed on October 18, 1938.

in front of the photoelectric cell. Good images were also obtained of prominences without any optical filter, i.e., in white light.

A number of the images showed features which apparently belong to the corona. Figure 4 shows such an image, it being identified by the test mentioned above as a flare or jet in the corona. This image was taken without any optical filter. It is one of 11 photographs that were taken over a period of more than two hours.

The major objectives of this phase of the work which were the development of an adequate instrument and the proving in of the method, have been achieved. The next phase of the investigation will be carried out under the more favorable conditions at the McDonald Observatory in Texas under the direction of Dr. Otto Struve.

[1] Lyot, B., *M. N. R. A. S.*, **99**, No. 8, 580 (1939).
[2] Skellett, A. M., *Proc. Nat. Acad. Sci.*, **20**, 461 (1934).
[3] The author is indebted to Dr. Cook and his associates for their able coöperation.
[4] *Bell System Tech. Jour.*, **19**, 2, 249 (1940).

A PATHOLOGICAL CASE IN THE NUMERICAL SOLUTION OF INTEGRAL EQUATIONS

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Consider an integral equation of the first kind

$$f(u) = \int_0^\infty H(v)K(v, u)dv \qquad (1)$$

where $f(u)$ and the kernel $K(v, u)$ are given, and $H(v)$ is to be determined from equation (1). Such equations are of frequent occurrence in physical problems,[1] where it often happens[2] that the analytical form of the kernel is known, but that $f(u)$ may be a function determined observationally at only a discrete set of values of u. $f(u)$ is then known only to within the errors of the observations and the question arises as to what accuracy $H(v)$ can be determined from equation (1). It is clear that $H(v)$ is subject to at least the uncertainties of $f(u)$. An additional uncertainty will arise from the approximate nature of the method of solution of the integral equation, whether this method be numerical or mechanical, and it is this type of uncertainty which will be discussed in the present note. One might be tempted to expect that, if a trial solution $H_1(v)$ when inserted in the right-hand side of equation (1) yields an $f_1(u)$ which differs from $f(u)$ by only a

few per cent, $H(v)$ is approximated to by $H_1(v)$ to the same degree of accuracy. The case to be cited presently shows that generally such an expectation is altogether unjustified. The integral equation is

$$f(u) = \frac{\sqrt{u + 1/3}}{(2u + 1)^2 - 4u\sqrt{u + 1/3}\sqrt{u + 1}} = \int^{\infty} \frac{H(v)dv}{(u + v)^{3/2}}. \quad (2)$$

For small values of u

$$f(u) = \frac{1}{\sqrt{3}} - \left(\frac{5}{2\sqrt{3}} - \frac{4}{3}\right)u - \ldots, \quad (3)$$

and for large values of u

$$f(u) \longrightarrow \frac{3}{4\sqrt{u}} - \frac{9}{16u^{3/2}} + \ldots. \quad (4)$$

For real values of u, $f(u)$ is a well-behaved function, as is shown in the second column of table 1. Equation (2) arises in the seismological problem of the motion of the surface of a uniform elastic half-space due to the sudden application of a localized normal stress p_{zz} at the surface. The variation with time of p_{zz} is represented by the Heaviside unit function $H(t)$ and its space localization is such that it is everywhere zero, except at the origin of coördinates where it becomes infinite in such a manner that

$$2\pi \int_0^{\infty} p_{zz}(r) r dr = Z, \quad (5)$$

Z being a (negative) constant. It is further assumed that Poisson's ratio is $1/4$, so that the velocity of the dilatational wave is $\sqrt{3}\,c$, where c is the velocity of the shear wave. This problem was originally solved by Lamb[3] by Fourier methods and the solution to be given presently for a pulse of the form $H(t)$ can be derived from his results. Lamb's method of solution is, however, very cumbersome and the author found that the solution of this problem, as well as of the more difficult problem for a layered half-space, can be obtained more expeditiously by operational methods. The details of the solution will be presented elsewhere and we shall give here only the results for the vertical component of motion w. Let r denote the distance on the surface from the origin, μ the coefficient of rigidity and

$$v = (ct/r)^2, \; w(t, r) = -(Z/\pi\mu r)H(v).$$

Then

$$H(v) = 0, \; v < 1/3,$$

$$H(v) = 3/16 - \frac{\sqrt{3}}{32\sqrt{v - 1/4}} + \frac{\sqrt{3\sqrt{3} - 5}}{32\sqrt{v - 1/4}(3 - \sqrt{3})} - \frac{\sqrt{3\sqrt{3} + 5}}{32\sqrt{1/4\,(3 + \sqrt{3}) - v}}, \; 1/3 < v < 1,$$

$$H(v) = {}^3/_8 - \frac{\sqrt{3\sqrt{3}+5}}{16\sqrt{{}^1/_4(3+\sqrt{3}) - v}}, \quad 1 < v < {}^1/_4(3+\sqrt{3}),$$

$$H(v) = {}^3/_8, \quad v > {}^1/_4(3+\sqrt{3}). \tag{6}$$

It can be verified by substitution in equation (2) that equation (6) is an exact solution. This solution is plotted as curve A in figure 1. It is seen

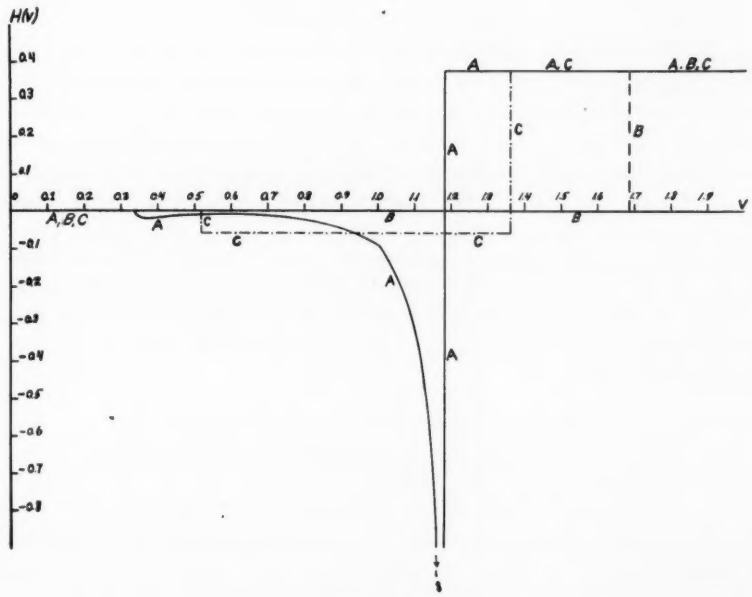

FIGURE 1

Curves A, B, C represent $H(v)$, $H_1(v)$ and $H_2(v)$, respectively. The associated f's are given by equations (2), (8) and (10).

that there is no motion until the arrival of the P wave at $v = {}^1/_3$; the shear wave, which is due to arrive at $v = 1$, is marked only by a discontinuity in slope. At $v_0 = 1.183$ the Rayleigh surface wave arrives and the amplitude becomes infinite as $(v_0 - v)^{-1/2}$. If t_0 is the arrival time of the Rayleigh wave, then for $t = t_0 - \delta$, where δ is small, $H(v)$ is proportional to $\sqrt{r/\delta}$. The infinity in w associated with the Rayleigh wave is therefore proportional to $r^{-1/2}$, which is characteristic of surface waves. Beyond v_0 the steady-state displacement is immediately obtained. From a seismological point of view, the chief interest lies in the nature of the discontinuities that are associated with the arrivals of P, S and Rayleigh waves, because the

recognition of these phases enables one to determine the velocities of medium.

Now returning to the integral equation (2) we see that a trial function $H_1(v)$ which is zero up to $v = a$ and is equal to A for $v > a$, yields an

$$f_1(u) = \frac{2A}{\sqrt{u + a}}. \tag{7}$$

Let us try a solution of this form and determine the constants A and a so that $f_1(u)$ will agree with $f(u)$ in the leading terms of the expansions (3) and (4). This yields

$$f_1(u) = \frac{3}{\sqrt{16u + 27}} \tag{8}$$

and the corresponding $H_1(v)$ is plotted as curve B in figure 1. As a seismogram, curve B is totally inadequate and yet, as is seen from the third column of table 1, the maximum deviation of $f_1(u)$ from $f(u)$ is only of the order of 2%. The situation is even more aggravated by the trial solution $H_2(v)$ which is given by

$$\begin{aligned} H_2(v) &= 0, \; v < 0.517787483 \\ H_2(v) &= -0.0612441866, \; 0.517787483 < v < 1.362107488, \\ H_2(v) &= {}^3/_8, \; v > 1.362107488. \end{aligned} \tag{9}$$

This function is plotted as curve C in figure 1. It was chosen so that the expansions of

$$f_2(u) = -\frac{0.122488373}{\sqrt{u + 0.517787483}} + \frac{0.872488373}{\sqrt{u + 1.362107488}} \tag{10}$$

agree with the four terms in the expansions of $f(u)$ which are given in equations (3) and (4). Curve C is again a bad sample of a seismogram for the medium in question. It yields a velocity of the P wave which is wrong by 25%; there is no sign of an S wave, and the phase corresponding to the Rayleigh wave arrives too late by 7%. In practice this phase would easily be mistaken for an S phase, because its amplitude diminishes with distance like r^{-1} instead of the $r^{-1/2}$ which is characteristic of Rayleigh waves, and this mistaken S wave would yield a shear velocity which is wrong by 17%. In spite of these deficiencies, $f_2(u)$ is seen from table 1 to differ from $f(u)$ by less than 0.1%.

TABLE 1

u	$f(u)$	$\frac{f_1(u)}{f(u)}$	$\frac{f_2(u)}{f(u)}$
0	0.5773503	1.000	1.000000
0.1	0.5656129	0.992	1.000184
0.4	0.5289999	0.981	1.000780
0.5	0.5175205	0.980	1.000857
1.0	0.4678638	0.978	1.000857
2.0	0.3984167	0.980	1.000555
3.0	0.3523105	0.983	1.000362
4.0	0.3190756	0.986	1.000251
6.0	0.2735410	0.989	1.000139

Summary.—An integral equation of the first kind arising in a seismological problem is cited for which a trial solution which is totally inacceptable reproduces the given function on the left-hand side to within 2%. A second trial solution, which is physically wrong by 25% and is otherwise deficient, reproduces the given function to within 0.1%.

[1] A good treatment of integral equations from the point of view of their applications will be found in Hamel, G., *Integralgleichungen*, Berlin, Springer (1937).

[2] Pekeris, C. L., *Gerl. Beit. z. Geoph.*, **41**, 192–202 (1934).

[3] Lamb, H., *Phil. Trans. Roy. Soc.*, (A), **203**, 1 (1904).

THE CONFORMAL THEORY OF CURVES

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In this note, we announce some of the principal results of a new conformal theory of curves in a Riemann space[1] V_n. The proofs of these results as well as the detailed conformal geometry of curves will be given elsewhere. In general, we assume that V_n is a Riemann space of class C^3. The precise hypotheses concerning existence, continuity and differentiability will appear in the paper which contains the proofs of these theorems.

Since every analytic curve in a V_2 is conformally equivalent to a straight line in the plane, there can be no conformal theory[2] of (single) curves in a V_n unless $n > 2$. We consider those properties of a curve which remain unchanged when the enveloping Riemann space V_n of dimensionality $n > 2$ undergoes any conformal mapping, not necessarily on itself. While the results obtained bear a close analogy to those which hold in the metric theory, in some cases the proofs are markedly different.

The principal tool is a new kind of tensor differentiation which has conformal meaning. This *conformal derivative* is dependent not only on

the metric of V_n but also on the curve C in V_n with respect to which the differentiation takes place. This dependence of conformal differentiation on a curve as well as the space is analogous to the similar dependence of parallel displacement of vectors in a general Riemann space. We find $n - 1$ differential *conformal curvatures* $J_1, J_2, \ldots, J_{n-1}$ and an integral *conformal arc length* S which are unchanged by any conformal transformation of V_n. This means that

THEOREM 1. *If* $V_n \longleftrightarrow \bar{V}_n$, $C \longleftrightarrow \bar{C}$ *by a conformal map, then a conformal arc length parameter* S *may be chosen so that the corresponding points of* C *and* $\bar{C}$ *have the same value of* S *and* $J_1, J_2, \ldots, J_{n-1}$ *are the same functions of* S *for* C *and* $\bar{C}$.

We prove the existence theorem

THEOREM 2. *In any* V_n, *a curve exists whose conformal curvatures are any arbitrary continuous functions of the conformal arc length.*

This curve is uniquely determined by a set of initial conditions which is found explicitly. In spaces conformal to a euclidean space, the following fundamental conformal equivalence theorem for curves exists which is the analogue of the (metric) congruence theorem:

THEOREM 3. *Let* C_1 *and* C_2 *be curves in the conformally euclidean spaces* ${}_{(1)}\bar{R}_n$ *and* ${}_{(2)}\bar{R}_n$, *respectively, whose conformal curvatures are the same functions of the conformal arc length. Then a conformal transformation exists so that* ${}_{(1)}\bar{R}_n \longleftrightarrow {}_{(2)}\bar{R}_n$ *and* $C_1 \longleftrightarrow C_2$.

The conformal curvatures have rather simple geometric properties if V_n is conformal to an Einstein space or, more particularly, to a euclidean space. For example, we mention the following theorems:

THEOREM 4. *The most general conformal differential invariant of a curve in an* $\bar{R}_n$ *is a function of the conformal curvatures and their derivatives with respect to a conformal arc length parameter. Conversely, every such function is a conformal differential invariant.*

Examples may be given to show that this theorem is untrue in spaces which are not conformally euclidean.

THEOREM 5. *The necessary and sufficient condition that* $J_\alpha \equiv 0$ $(0 < \alpha < n - 1)$ *along a curve* C *in an* $\bar{R}_n$ *is that* C *be conformally equivalent to a curve in* R_n *whose* $(\alpha + 1)$*st metric curvature is identically zero.*

As an important special case, this theory includes the "natural geometry of curves" (according to the viewpoint of Cesàro, Pick and Kowalewski) in R_n under the continuous group of conformal mappings of R_n on itself. In particular, the curves along which each conformal curvature is equal to a constant are the paths of the group.

If $n = 2$, the above theory applies if the conformal mappings are restricted to transformations (applied to surfaces of constant curvature) which are similar to and include the inversive transformations of the plane. In this case, the present theory includes the inversive theory of

plane curves developed by Mullins, Liebmann, Kubota, Morley and Patterson who used different methods than those employed here.

The methods and results of this note may also be used to develop a theory of curves in a conformal Riemann space. From this point of view, this theory is related to the work of Hlavatý, Sasaki, Yano and others on curves in a conformal Riemann space.

A preliminary investigation tends to show that a conformal theory similar to that outlined above exists for any subspace in V_n. At points of a subspace, it is possible to define a "conformal derivative" and to arrive at a sequence of normal vector spaces and fundamental forms which are unchanged by conformal transformations of V_n.

[1] We denote an n-dimensional Riemann space and euclidean space by V_n and R_n, respectively. Spaces conformal to V_n and R_n are denoted by $\overline{V}_n$ and $\overline{R}_n$, respectively.

[2] The general conformal geometry for two dimensions (equivalent to the theory of analytic functions of a complex variable), developed by Kasner since 1910, deals with invariants, absolute and relative, of two or more curves (horn angles and trihornometry). See *Trans. Amer. Math. Soc.*, **44**, 25–31 (1938), and these PROCEEDINGS, **23**, 337–341 (1937).